University of Alberta

Hulless Barley Starches: Isolation and Chemical Modification

by

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A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of

Master of Science

in

Food Science and Technology

Department of Agricultural, Food and Nutritional Science

Edmonton, Alberta

Spring, 2008

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ISBN: 978-0-494-45811-2
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Abstract

An improved protocol for barley grain fractionation and starch purification was investigated. Regular, waxy and high amylose barley grains were fractionated into starch isolate, fiber and protein concentrates and grain component mass balance was determined. Starch isolates primarily had large granule with high purity (>98%), and their yield ranged from 22 to 39% (flour dry-weight basis). The second study focused on chemical modifications of barley and maize starches from different genotypes. It was hypothesized that partial acid treatment to native starches would improve their chemical reactivity towards phosphorylation and cationization. Data suggested that acid treatment, depending on acid strength and treatment duration, would significantly enhance phosphorylation at first and then reduce. Acid treatment did not influence cationization. Physicochemical properties of the control and acid treated starches suggested that changes caused by acid on the extent of intermolecular associations in the native starch amorphous regions, may be responsible for the changes in chemical reactivity.

To my beloved Wife Shuxiang, my Dad, Mom, and my daughters, Yiyin and Kristina

for their love and encouragement.

ACKNOWLEDGEMENTS

I would like to express my most sincere thanks to my supervisor, Dr. Thava Vasanthan, for his valuable support, insightful advice, constant encouragement and patient guidance given at all stages in my study. I would also like to express my genuine gratitude to Dr. Feral Temelli for her insightful direction and priceless support through all these years. Thanks to Dr. Phillip Choi for coming here and serving as an external examiner.

Special thanks to Dr. Ratnajothi Hoover (Memorial University of Newfoundland), for his input into this research and critical review of my manuscripts for publication and thesis. Thanks to Dr. Gordon Grant for his suggestions on thesis preparation. Thanks to all the friends and colleagues who work in the lab, Dr. Li, Dr. Faraj, Dr. Ghotra, Dr. Khan, Judy, Vivian, Mei, Haiyan, Nagul, Arun, Lisa, Anchalee, Dr. Nakano, Yuheng, for all their friendly assistance and support given throughout this study.

I appreciate the financial support from the Natural Science and Engineering Research Council (NSERC) of Canada to the program of Dr. T. Vasanthan which supported my research. I also like to acknowledge the travel grants from the Graduate Students' Association, the Faculty of Graduate studies and Research, Department of Agricultural, Food and Nutritional Science, University of Alberta.

Thanks to all staff and students in our department for their routine support and creating a pleasant environment in which I work.

Finally, I reserve my deepest gratitude to my wife Shuxiang, my Dad and Mom. Their constant support, unselfish love and care make me succeed.

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List of Abbreviations

ΔH	Enthalpy
AACC	American Association of Cereal Chemists
AFM	Atomic force microscopy
AML	Amylose leaching
BOD	Biological oxygen demand
BV	Blue value
CDC	Crop Development Centre
CL	Chain length
Da	Daltons
db	Dry weight basis
DP	Degree of polymerization
DS	Degree of substitution
DSC	Differential scanning calorimetry
FAM	Lipid-free amylose
FAO	Food and Agricultural Organization of the United Nations
FDA	United States Food and Drug Administration
GLM	General linear model
HA	High amylose starch
HB	Hulless barley
HPLC	High performance liquid chromatography
IA	Iodine affinity
LAM	Lipid complexed amylose
LPL	Lysophosphatidyl choline
MALDI-MS	Matrix-assisted laser desorption/ionization-mass spectrometry
MHA	Maize high amylose starch
MRA	Maize regular starch
Mw	Molecular weight
MWX	Maize waxy starch
NC	Number of chains per molecule
NMR	Nuclear magnetic resonance
RA	Regular starch

RC	Relative crystallinity
Rg	Radii of gyration
SEM	Scanning electron microscopy
SF	Swelling factor
STMP	Sodium trimetaphosphate
STPP	Sodium tripolyphosphate
T_c	Conclusion temperature
TEM	Transmission electron microscopy
T_o	Onset temperature
T_p	Peak temperature
WX	Waxy starch

Chapter 1

Introduction and objectives

1.1 Introduction

Cereals are important economic commodities worldwide. Barley is the fourth most important cereal crop of the world after wheat, maize and rice. Canada is the second largest barley producer in the world with the production of 13.5 million metric tons (FAO, 2006). Barley is principally used as feed for animals, in the form of barley meal, or as grain for malting and brewing in manufacturing of beer and whiskey. However, the barley crop may be considered relatively under-utilized with regard to its potential use as an ingredient in processed human foods. Although hulless barley (HB) has a long history of being utilized in many countries, its current use in the human diet is very limited. Recent research findings on the health promoting properties of beta-glucan, mainly from barley and oat, have generated considerable interest in barley (Brennan and Cleary, 2005). Furthermore, the US Food and Drug Administration (US FDA) now allows barley products which include whole grain barley and barley-containing products to claim reduction in risk of coronary heart disease this is mainly due to its high soluble fiber-beta-glucan content (US Department of Health and Human Services, Food and Drug Administration, 2006). That should encourage more commercial development of barley food products and industry interest is fast growing for the fractionation of barley grain to concentrate beta-glucan. When beta-glucan is concentrated from barley meal/flour, crude starch is obtained as a major by-product. Although HB starch has significant potential in the starch industry and has properties comparable to those of maize starch (Vasanthan

and Bhatty, 1996), it is not extensively used in a large number of food and other non-food industrial applications in North America.

Currently across the world, maize and potato starches are commonly used in many food and non-food applications. Native maize and potato starches are often chemically modified to overcome one or more of their shortcomings in functionality such as poor shear/thermal stability, undesirable texture, and poor processing properties. These functional limitations lessen the usefulness or prevent the utilization of native starches in food applications. Cationization and phosphorylation are two common derivatization methods of chemical modification. In papermaking, cationic starches have found large-scale use as wet-end additives, surface size, and coating binders. The use of cationic starches can increase paper strength and decrease biological oxygen demand (BOD) of paper mill effluent. Phosphorylated starch and phosphorylated distarch may be used as thickeners and stabilizers for food systems. These create reasonably clear and stable dispersions, which are suitable for thickening food systems that are stable through freezing and thawing.

Randomly distributed pinholes or pores have been observed on the surface of maize, sorghum and millet starch granules (Fannon et al., 1992). These pinholes are exterior openings to internal channels that penetrate the granule interior (Fannon et al., 2004). The pinholes, together with internal channels, are potentially increasing the granule surface area available for chemical and enzymatic reactions (Huber and BeMiller, 1997, 1999, 2001; Fannon et al., 2004; Gray and BeMiller, 2004; Benmoussa et al., 2006). Li et al. (2004) also observed the presence of pin holes on the surface and internal

channels in some HB starch granules. Acid can permeate the starch granule and erode these pores and holes to open them more (Jayakody and Hoover, 2002).

It is hypothesized that the changes in the starch granular ultra-structure, caused by partial acid treatment, can influence the extent of chemical modification. The hypothesis assumes that starch hydrolysis will increase the porosity and permeability of starch granules, and thus increase the reactivity of starches towards chemical reagents. This is critical from an industrial perspective, since there is a large cost of reactant removal and recovery that needs to be balanced with degree of chemical substitution. Also, the proposed investigation will benefit the starch research community by improving the understanding of the relationships between starch granular ultra-structure and reactivity. No research to date has evaluated the effect of partial acid treatment to starch and subsequent changes in its ultra-structure and chemical reactivity.

1.2 Thesis Objectives

The objectives of this thesis are:

1. To develop a new and efficient approach to HB barley starch isolation and purification, and to characterize the isolated starches in terms of their basic physicochemical properties such as granule surface characteristics (by scanning electron microscopy), relative crystallinity (by X-ray diffractometry), pasting properties (by Brabender amylography) and the gelatinization temperature (by differential scanning calorimetry).

2. To investigate how partial acid (1 N and 2.2 N HCl) pre-treatment of barley and maize starches influence their reactivity towards phosphorylation and cationization.

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Chapter 2

Literature review

2.1 Barley grain

2.1.1 Introduction

Barley (*Hordeum vulgare*) is a grass belonging to the family *Poaceae*, the tribe *Triticeae* and the genus *Hordeum* (Nilan and Ullrich, 1993). As one of the oldest cereals in recorded history, barley was domesticated about 17, 000 years ago along the Nile River Valley in Egypt (Nilan and Ullrich, 1993). As the major food grain in many parts of the world, this grain was used as a staple food in the Near East several thousand years ago and was the chief form of nourishment for the Greeks in Homeric times (800 B.C.).

Barley is a versatile crop and can be grown under a wide range of environmental conditions. Cultivated varieties are of the six-row and two-row type, depending on the number of fertile spikelets on the rachis. The two-row barley is mostly used for malting/brewing and food processing, while the six-row type has been utilized mainly as livestock feed (Bhatty, 1993). As they have evolved in nature, barley grains are covered with hulls or husks, where the lemma and palea adhere to the caryopsis and do not thresh freely. However, hulless barley (HB) has been utilized in many countries since ancient historical time. Recently, a series of new HB genotypes have been developed through a breeding program at the Crop Development Centre, University of Saskatchewan. HB is popular as food or feed grain because it requires no pearling and thus retains nutrients that would otherwise be lost during processing (Bhatty and Rossnagel, 1981; Newman and Newman, 1991; Bhatty, 1999).

2.1.2 Structure and composition

A longitudinal section of a barley grain is illustrated in Figure 2.1 (Newman and Newman, 1991). The outermost part of hulled barley is the hull or husk, consisting of two leaf-like structures, the lemma and palea, which completely cover the grain. The hull or husk is rich in cellulose, insoluble arabinoxylans, lignin, polyphenols and minerals (MacGregor, 1998), and constitutes up to 10-13% of the dry weight of barley grain. The hull covers the caryopsis (kernel), which includes the pericarp, seed coat and endosperm. The pericarp contains cellulose, lignin and arabinoxylans. The endosperm which contributes to 75-80% of the total kernel weight is rich in starch, which is embedded in a protein matrix (MacGregor and Fincher, 1993). The cell walls in the endosperm are mainly composed of beta-glucan (70-75%), arabinoxylans (20-25%) and protein (5-6%) with minor amounts of glucomannans, cellulose and phenolic compounds (Jadhav et al., 1998; MacGregor, 1998). The outermost layer of the endosperm, called the aleurone layer, is comprised of arabinoxylans (60%), beta-glucan (22%) and proteins (16%), and contributes to about 5-10% of the total kernel weight. The embryo (2-4% kernel weight) is rich in lipids (13-17%), protein and amino acids (34%), sucrose and raffinose (5-10%), arabinoxylans, cellulose and pectin (8-10%) and ash (2-10%) (MacGregor and Fincher, 1993).

The chemical composition of hulled and hulless barley grains is presented in Table 2.1 (Oscarsson et al., 1996; Xue et al., 1997; Bhatty and Rossnagel, 1998; Andersson et al., 1999; Li et al., 2001a). The major components are starch (52-72%), protein (9-14%), lipids (2-3%), and non-starch polysaccharides (MacGregor, 1998) which contribute the major portion of the total dietary fiber, including cellulose/lignin (4-6%),

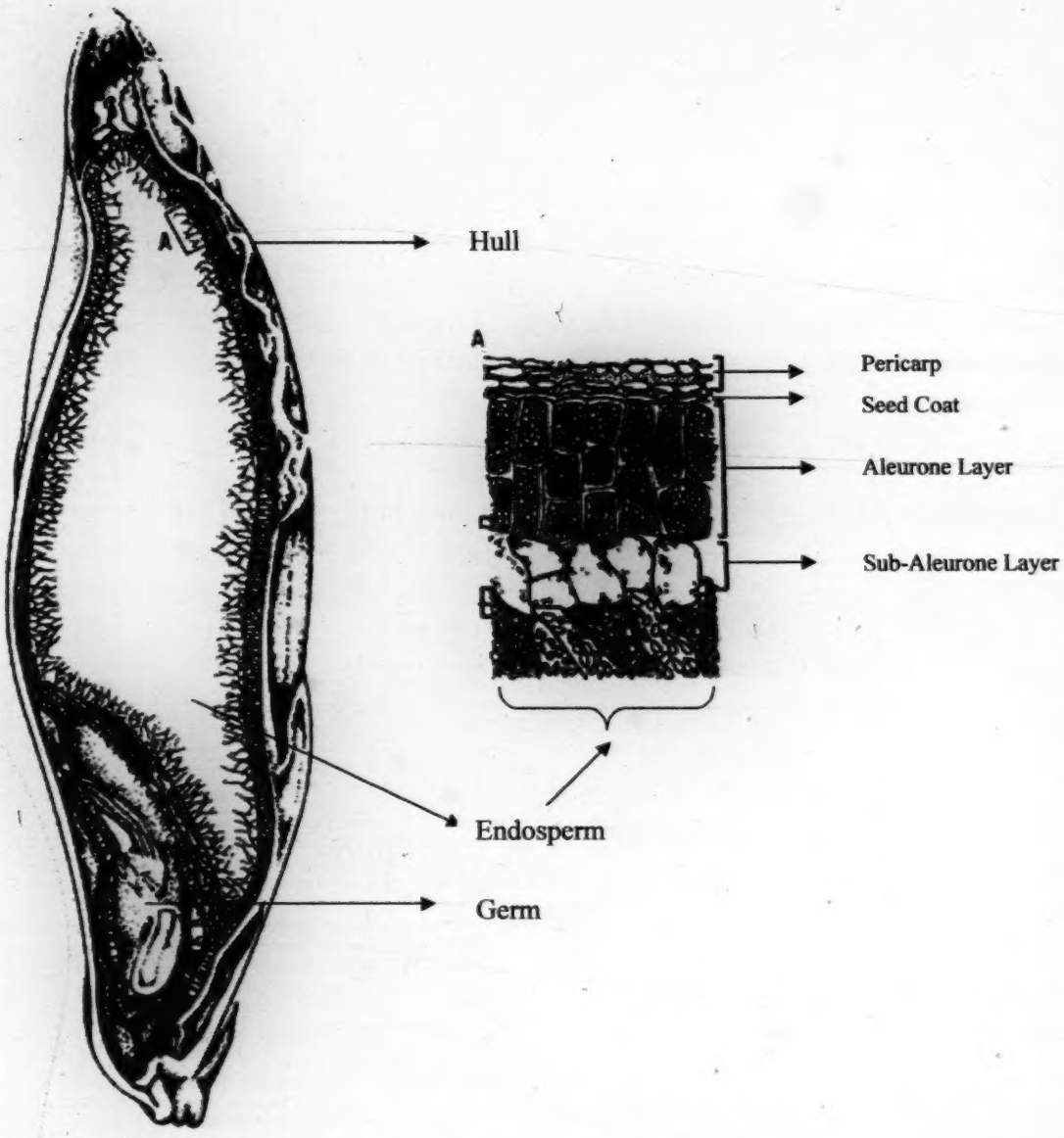

Figure 2.1 A longitudinal section of a barley grain (adapted from Newman and Newman (1991) with minor modification).

Table 2.1 Average chemical composition of hulled and hulless barley grains

Component	Hulled (%, db)			Hulless (%, db)				
Starch	52.1-63.8	49.4-63.1	53.7	23.9-64.4	60.1-73.8	56.0-64.7	49.4-66.2	59.7
Protein	8.7-10.5	9.3-15.5	15.9	11.3-18.1	12.8-17.8	11.5-14.2	10.6-21.9	16.5
Lipid	2.2-3.5	2.1-3.1	2.1-3.1	2.9-6.2	-	4.7-6.8	2.1-3.7	-
Dietary fiber	18.9-23.8	18.1-27.5	18.6	13.5-34.5	11.0-16.6	-	13.6-20.2	13.8
Cellulose	3.5-4.7	3.1-7.0	4.1	1.4-4.1	-	-	1.7-5.0	2.0
Lignin	1.4-1.7	1.0-1.9	2.0	0.7-1.1	-	-	0.5-0.9	9.0
Arabinoxylans	7.5-9.0	0.4-0.7	6.5	4.8-12.2	-	-	0.7-0.9	4.5
Beta-glucan	2.8-6.9	3.8-6.3	5.2	4.6-14.9	4.1-8.0	3.7-7.7	4.7-7.9	5.6
Ash	2.3-2.6	1.9-2.3	2.8	2.0-2.3	1.8-2.2	1.8-2.4	1.3-2.1	2.1
Reference	Andersson et al. (1999)	Oscarsson et al. (1996)	Xue et al. (1997)	Andersson et al. (1999)	Bhatty and Rossnagel (1998)	Li et al. (2001a)	Oscarsson et al. (1996)	Xue et al. (1997)

beta-glucan (3-6%), hemicellulose/pentosans (mainly arabinoxylans) (4-7%), and fructans (0.2-0.9%). The chemical composition of barley grain differs with variety and growing conditions. In general, HB contains proportionally more protein, starch, and total and soluble beta-glucan than hulled barley (Bhatty, 1999).

2.1.3 Production and utilization

Barley is the fourth major cereal crop grown worldwide, after wheat, maize and rice. The world production of barley was 135 million metric tons in 2005 (FAO, 2006). Some of the major barley producing countries are Canada, United States, Australia, France, Spain, Finland, Denmark, Germany, Turkey, China, England and the Russian Federation. Canada is the largest barley producer in the American continent with production of 13.5 million metric tons, accounting for 10% of the total world production (FAO, 2006). In Canada, the Prairie Provinces, Alberta and Saskatchewan contributed up to 88% of the national barley production (Jadhav et al., 1998).

Of the total world barley production, about 50% of barley is used as animal feed, 30% for malt to produce beer, whiskey and syrup, 10% for seed and 10% for food (McKenna, 2006). Utilization of barley in Canada is mainly in the industries of feed (75%), malting and brewing (20%) and the remaining (5-6%) has been for other human food uses (Alberta Agriculture, Food and Rural Development, 2004). Recent interest in HB utilization in the food industry developed largely due to its high beta-glucan content, which was more predominant in the waxy cultivars (Bhatty, 1999). Some researchers (Bhatty et al., 1997; Zheng et al., 1998) also reported a HB line containing zero-amylose starch that showed higher freeze-thaw stability than waxy HB (~5% amylose) starch.

Furthermore, the US Food and Drug Administration (US FDA) now allows barley products which include whole grain barley and barley-containing products to claim reduction in risk of coronary heart disease (US Department of Health and Human Services, Food and Drug Administration, 2006). That should encourage more commercial development of barley food products.

Value-added processing in terms of fractionation of valuable components to be used in foods, pharmaceuticals, cosmetics and biotechnology will help diversify barley utilization. A recent invention relating to fractionation of barley and oat grains into valuable components, especially beta-glucan, was reported (Vasanthan et al., 2004). The process is particularly effective in concentrating beta-glucan in a state close to its native form. The crude starch separated as a by-product during beta-glucan concentration needs further research to find industrial uses.

2.2 Starch

Isolated starch generally contains polysaccharides (85-90%, w/w), moisture (10-15%, w/w) and small amount of proteins, lipids and minerals (Tester, 1997b). Amylose and amylopectin are two types of alpha-glucan, which are found in starch granules and represent approximately 98-99% of the dry weight of starch. The ratio of the two polysaccharides varies according to the botanical origin of the starch. The comparison of basic characteristics of these two polymers is presented in Table 2.2.

Amylose is essentially a linear α-1, 4-D-glucan chain with a low degree (0.27-0.68%) of α-1, 6 linked branch points [see Figure 2.2 (A)]. The molecular weight and

Table 2.2 General characteristics of amylose and amylopectin [1]

Property	Amylose	Amylopectin
Branch linkage (%)	0.2-0.7	4.0-5.5
Degree of polymerization	700-5000	10^4-10^5
Molecular weight, Dalton	10^5-10^6	10^7-10^9
Average chain length	100-550	17-31
Iodine affinity, g/100g	19-20.5	0-1.2
Blue value	1.2-1.6	0-0.2
β- Amylolysis limit, %	70-95	55-60

[1] Reference: Hizukuri et al. (1981, 1983, 1997, 2006) and Yoo and Jane (2002)

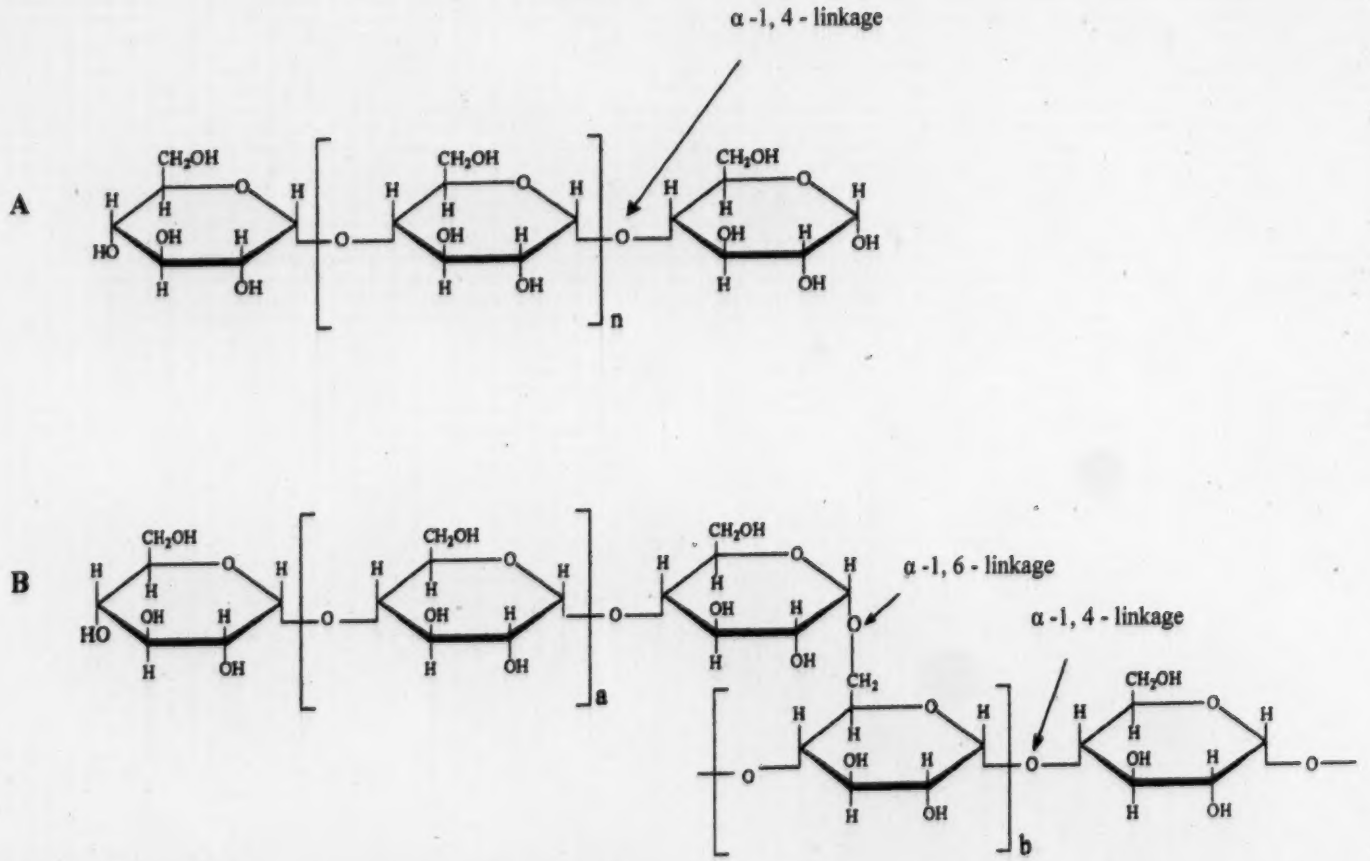

Figure 2.2 The structure of (A) amylose: average chain length n = 1000 and (B) amylopectin: average chains a = 12-23 and b= 20-30 (adapted from Tester et al. (2004) with minor modification).

degree of polymerization (DP) of amylose are usually in the range of 10^5-10^6 Da and 700-5000 anhydro-glucose units, respectively (Hizukuri et al., 1981, 1997). The presence of branched chains does not significantly influence the general properties of amylose which in neutral, aqueous solution behaves as a random, flexible coil due to the natural twist present in the chair conformation of the glucose units. The linear portion of amylose can form complexes with various ligands, such as lipids resulting in a V-polymorph X-ray pattern (Figure 2.3).

Amylopectin is a highly branched molecule composed of thousands of linear α-1, 4-D glucan unit chains and 4-5.5% of α-1, 6-glycosidic bonds (Hizukuri et al., 1997) (Figure 2.2 (B)). The average chain length and average molecular weight of amylopectin is 17-31 anhydro-glucose units (Hizukuri et al., 1983) and 7.0×10^7-5.7×10^9 Da (Yoo and Jane, 2002), respectively. Consequently, the amylopectin molecule is relatively compact and does not form the characteristic dark blue color complex with polyiodine ions in aqueous solution as amylose does.

The most widely accepted model for the molecular arrangement of amylopectin is derived from the cluster model of French (1972) and Robin et al. (1974), refined by Hizukuri (1986) (Figure 2.4). It is proposed that amylopectin molecules contain three types of unit chains. The short or A-chains are unbranched and linked to the molecule through their reducing end group by α-(1→ 6) glycosidic bonds. The long or B-chains are similarly joined to the molecule but carry one or more A-chains. There is only one C-chain, which carries the reducing group of the molecule. The unit chains are thought to concentrate in clusters, with the A and B_1-chains occurring within individual cluster. Hizukuri (1986) introduced the concept of B_1, B_2, B_3, etc, where B_2-chains join two

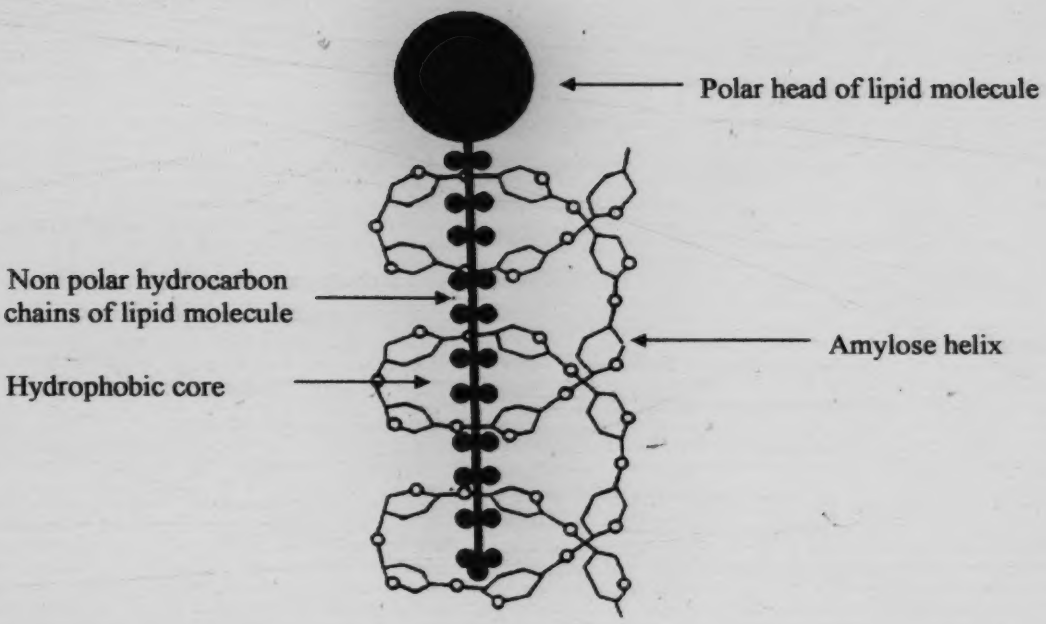

Figure 2.3 Schematic representation of amylose-lipid complex (adapted from Carlson et al. (1979) with minor modification).

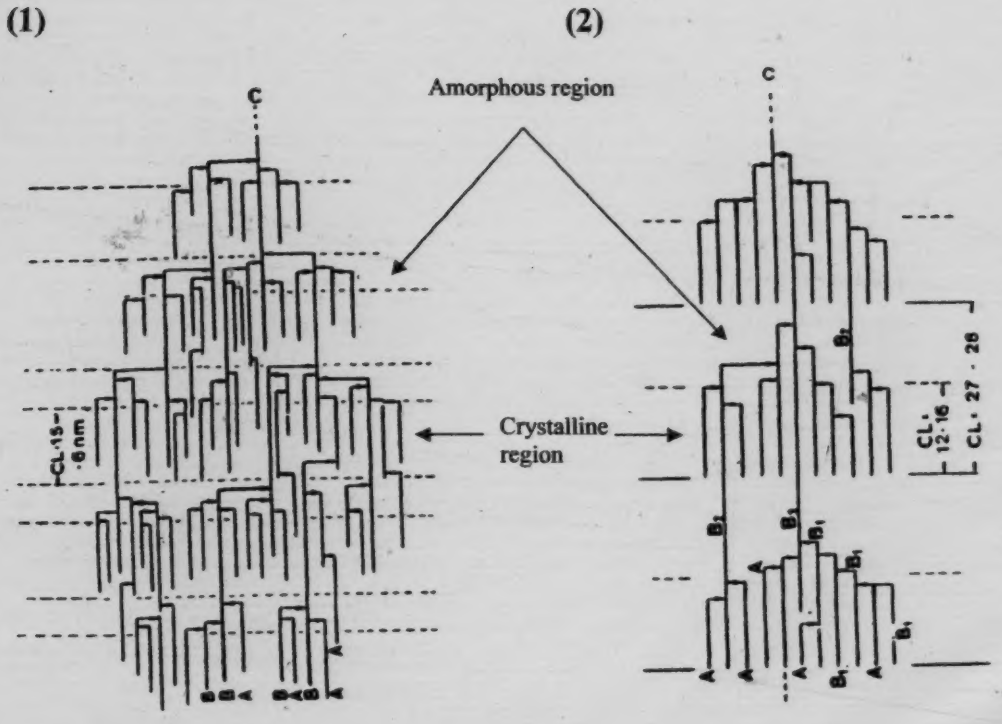

Figure 2.4 Amylopectin cluster models as proposed by (1) Robin et al. (1974) and (2) Hizukuri (1986). A: outermost branches; B: inner branches and C: the chain that carries the only reducing group; CL=chain length.

clusters while B_3 joins three clusters. The A and B_1 chains are the most exterior and form double helices (and potentially crystallites) within native granules (Tester et al., 2004). The double helices are formed with two neighboring short chains fitting together compactly, with the hydrophobic parts of the opposed glucose units in close contact inside the structure, and hydroxyl groups at the outside of the double helix resulting in strong inter-chain hydrogen bonding (helical order or short-range order).

Starch granules have crystalline and amorphous regions. The level of crystallinity in granular starch is 15-51%, with an average of about 35% (Tester et al., 2004). The origin of crystallinity is understood to be due to the intertwining of the outer chains of amylopectin in the form of double helices (Tester et al., 2004). Adjacent double helices give rise to regular three-dimensional patterns. The extent of crystallinity is influenced by: 1) the amount of double helices that are organized into a crystalline array; 2) crystallite size, 3) amylose content; and 4) moisture content.

The crystalline patterns within starch granules from various botanical sources are known and have well defined X-ray diffraction patterns that have been classified as A- (cereals), B- (root, tubers) and C- (legumes) type polymorphs. Figure 2.5 clearly shows the differences between these three types. The A- and B-types are believed to be independent, whereas the C-type is a mixture of A- and B-type crystallites in varying proportions (Hizukuri, 1996). The differences between the two X-ray polymorphs of A- and B- relate to the packing of double helices in the crystal unit cell and the quantity of water molecules stabilizing these double helices (Imberty and Perez, 1988; Imberty et al., 1988).

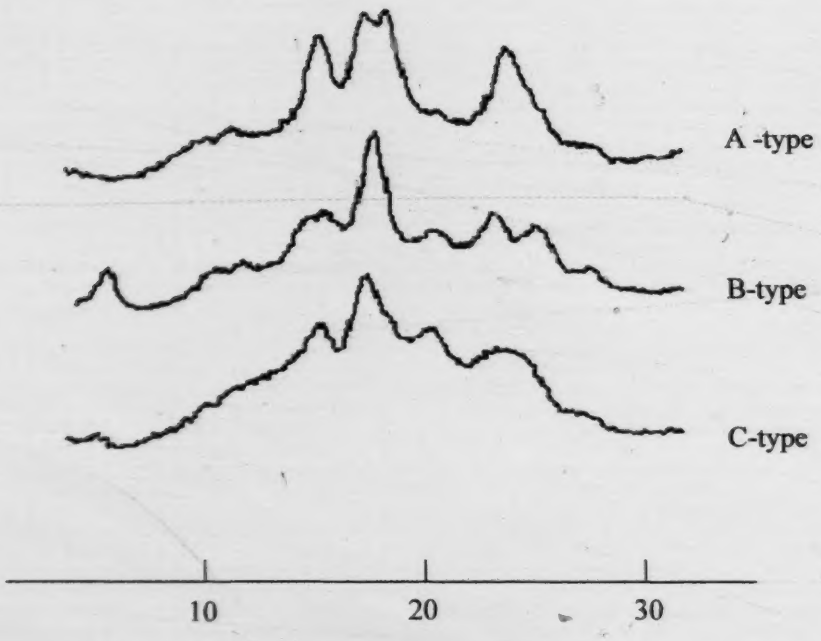

Figure 2.5 X-ray diffraction patterns of A-, B-, C-type starches (adapted from Zobel (1988) and Cheetham and Tao (1998)). A-type pattern shows three strong peaks at 2Θ 15° (d=5.5-6.0Å), 17° (d=5.2Å), and 23° (d=3.9Å). B-type pattern shows strong peaks at 5.3-5.6° (d=15.8-16.7Å) and 17° (d=5.2-5.3Å), and medium intensity peaks at 14.4-15.0° (d=5.9-6.1Å), 22° (d=4.0Å) and 24° (d=3.7Å). C-type pattern is the same as that of A-type except for the addition of the medium to strong peak at about 5.2-5.5° (d=16.0-16.9Å). The d-spacing at 4.4-4.6Å (2Θ=20°) is characteristic of amylose-lipid complex (Zobel, 1988).

Amylopectin is the main crystalline component of the starch granule. Amylose does not have any significant effect on the crystallinity in normal and waxy starches, which display strong birefringence (French, 1972; Banks and Greenwood, 1975; Blanshard, 1987; Zobel, 1988; Hoover, 2001). However, the amylose may contribute significantly to the crystallinity of high amylose starches (Banks and Greenwood, 1975; Tester et al., 2004).

How amylose and amylopectin are packed in the starch granule is not fully understood. Some researchers (Blanshard, 1987; Zobel, 1992) have shown that amylose is located in bundles between amylopectin clusters; however, others (Jane et al., 1992; Kasemsuwan and Jane, 1994) have shown that amylose is interspersed as individual molecules both in the amorphous and crystalline regions of the granule. Jenkins and Donald (1995) suggested that amylose and amylopectin are co-crystallized or amylose penetrates into the amorphous regions of the clusters to disrupt the packing of the amylopectin double helices within the crystalline lamella. Gallant et al. (1997) have suggested that amylose may occur almost everywhere in granules. Jane (2006) postulated that amylose is oriented side by side with amylopectin and concentrated at the periphery of the starch granule. This may enhance the interaction and association between amylose and amylopectin on the starch granule surface, therefore inhibiting enzyme hydrolysis.

The fine structure of starch granules is not fully understood. Tang et al. (2006) developed the concept of blocklets and proposed a model based on this basic constructing unit (Figure 2.6). The idea of a blocklet structure for starch granules was suggested by Nägeli as early as 1858 and re-emphasized by Gallant et al. (1997). Recently, atomic force microscopy (AFM) studies of starch granule surfaces and of the internal structure of

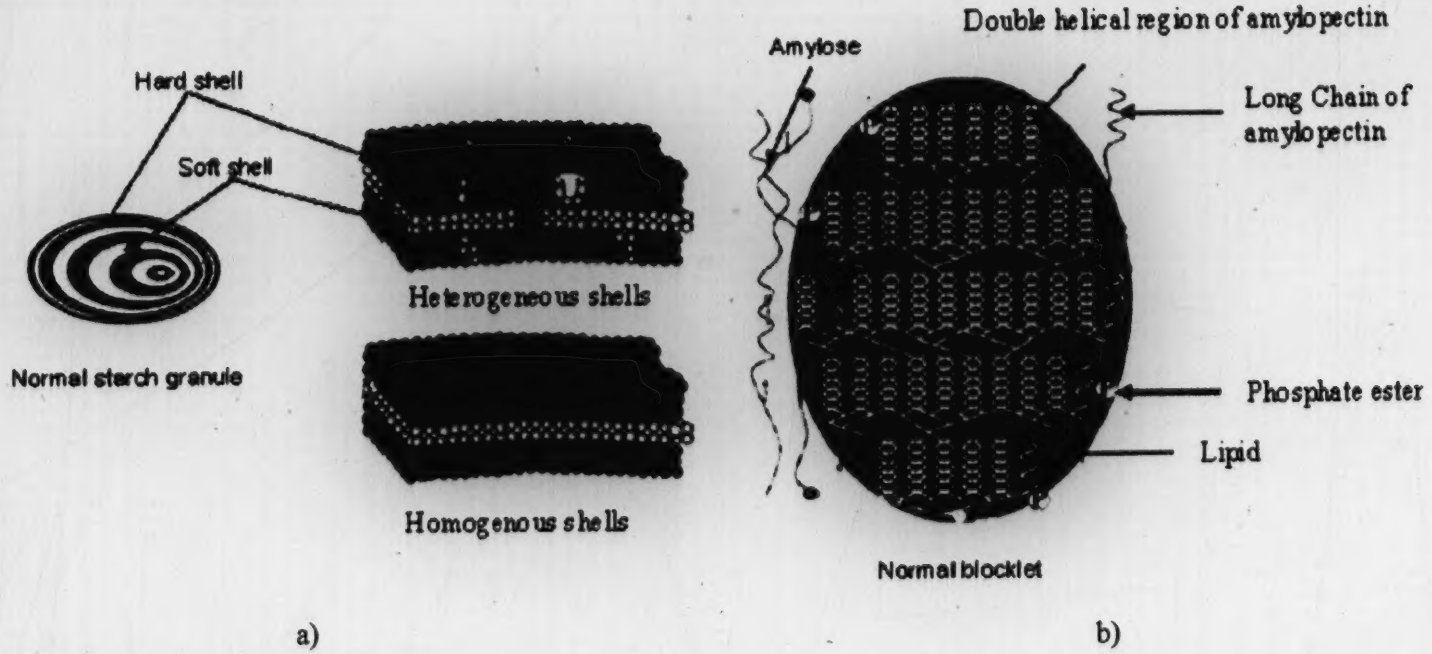

Figure 2.6 Scheme of starch granule structure (a) and scheme of normal blocklet structure in starch granules (b) (adapted from Tang et al. (2006) with minor modification)

starch granules have added weight to this idea (Baker et al., 2001; Ridout et al., 2002, 2003; Szymonska and Krok, 2003; Ridout et al., 2004a, 2004b; Ayoub et al., 2006; Liu et al., 2007). According to Tang et al. (2006), the blocklet is a basic construction unit, which consists of several amylopectin molecules and be classified as normal and defective. The defective blocklets are unfavorable to crystallization and produced when lower branching molecules are put in a blocklet. The normal and defective blocklets can build the heterogeneous and homogenous shells of the starch granules. As for the homogeneous shells like in quinoa, potato and amylomaize, the stream of the normal blocklets and the defective blocklets are continuous. Therefore, the shells have consistent resistance to the enzymes. However, in heterogeneous shell, both the hard shells and soft shells are not constant structures and soft shells penetrate into hard shells. Because the defective blocklets gather loosely, the zones are easy to shed off, and form pores in the surface of the starch granules during processing or storage resulting in weak resistance to enzymes (Tang et al., 2006).

2.3 Barley starch

2.3.1 Starch isolation and purification

Various laboratory methods involving grain steeping, blending followed by screening, deproteinization, and recovery of starch by centrifugation have been used for isolation of starch from barley grains. Compared to maize starches, barley starch isolation by wet extraction presents a special difficulty. Beta-glucans (1→3 and 1→4), major cell-wall polysaccharides of barley, produce high viscosities in aqueous solutions and impair

separation of starch by screening and subsequent centrifugation (Zheng and Bhatty, 1998).

One of the earliest methods of barley starch isolation was proposed by Greenwood and co-workers (Greenwood and Thomson, 1959; Adkins and Greenwood, 1966; Banks et al., 1973). They used aqueous mercuric chloride solution (0.01 M) for steeping, nylon mesh (75 μm) for screening and repeated toluene washing for deproteinization. McDonald and Stark (1988) outlined a process for barley starch extraction that was based on a method developed by Morrison et al. (1984). The scheme involved acid (pH = 2) steeping of cracked grains followed by neutralization, gentle grinding, and screening through a nylon mesh (75 μm). The crude starch was recovered by centrifugation of the filtrate and by scraping of the brown proteinous layer. Small starch granules in the brown proteinous layer were recovered by protease treatment followed by toluene shaking, and then added back to the main stock starch. South and Morrison (1990) isolated starch from waxy and non-waxy barley grains by steeping in water, maceration and then purification by centrifugation in the presence of cesium chloride (CsCl). CsCl was used to remove cellular material, storage proteins and some starch granule surface proteins. They obtained yields of 36 and 44%, respectively for waxy and non-waxy barley starches. Zheng and Bhatty (1998) isolated starch from hulless barley grains (varying widely in amylose content) using an enzyme-assisted wet separation process. The enzyme cocktail consisting of cellulase, endo (1→3), (1→4)-β-D-glucanase and xylanase separated starch, protein, beta-glucan, bran and tailings resulting in starch yields in the range of 44 - 54% of total dry matter. The purity of the starch was 98.0%. Li et al. (2001a) isolated starches from 10 cultivars of hulless (HB) barley grains

following the wet-milling procedure of Wu et al. (1979). They reported average yields and extraction efficiencies of 44.4% and 70.9%, respectively. Waxy HB genotypes with high beta-glucan content gave low starch yields (42.1%) and extraction efficiencies (68.0%). Isolated starches contained 95-98% starch with beta-glucan contents in the range of 0.01 to 0.06%. Vasanthan and Bhatty (1995) reported a procedure for pin milling and air-classification of hulless barley to obtain fractions rich in starch, protein and beta-glucan. The starch-rich fraction, containing 78% starch, was subjected to a wet processing method to obtain pure starch that contained mainly large granules. The starch yield from this dry- and wet-processing was ~34%, which is similar to that of the conventional wet milling procedure.

Barley starch has been shown to exist in two clearly defined populations (Vasanthan and Bhatty, 1995) large, lenticular (A-type) and small, irregular shaped (B-type) granules (Figure 2.7). The A and B type granules have been fractionated by decantation (Tang et al., 2000) and by pin-milling and air-classification (Vasanthan and Bhatty, 1995).

2.3.2 Chemical composition of barley starch

2.3.2.1 Carbohydrate components

The amylose content of barley starches ranges from 0 to 46% (Vasanthan and Bhatty, 1995; Zheng et al., 1998; Yoshimoto et al., 2000; Yoshimoto et al., 2002; Waduge et al., 2006). The corresponding value for maize starches ranges from 0.62 to 68% (Morrison et al, 1984; Hizukuri, 1996; Li et al., 2001). Morrison et al. (1993b) and Tester and Morrison (1993) have shown that amylose in barley starches exists partially as

lipid complexed amylose (L·AM) with a lysophosphatidyl choline (LPL) to L·AM ratio of 1:7 and partially as lipid-free amylose (F·AM). These authors have shown that L·AM and F·AM in waxy barley starches range from 0.8-4.0% and 0.9–6.4%, respectively. On the other hand, the corresponding values for non-waxy barley starches are 5.1–7.2% and 23.1–25.9% respectively. In high and low amylose barley starches, L·AM is 12.1% and 7.1%, respectively in the small granules, and 4.1% and 3.2%, respectively in the large granules (Vasanthan and Bhatty, 1996). The number average degree of polymerization (DPn), average chain length ($\overline{CL}$), average number of chains per molecule (NC), average molecular weight (Mw) and radii of gyration (Rg), the β-amylolysis limit, iodine affinity and limiting viscosity number of barley amylose are summarized in Table 2.3, and maize starches are used for comparison.

Properties of maize and barley amylopectin are summarized in Table 2.4. The length of the amylopectin B chains (Salomonsson and Sundberg, 1994), size of the crystalline lamella (Jenkins and Donald, 1995) and formation of amylose-lipid complexes (Vasanthan and Bhatty, 1996) increase with an increase in barley amylose content. Debranched amylopectins of normal, waxy and high amylose barley starches exhibit similar chain length distribution profiles, all showing trimodal distributions of short chains (mostly A chains), intermediate chains (mostly B1 and some A chains) and long chains (mostly B2 and B3 chains) (MacGregor and Morgan, 1984; Tester et al. 1991; Song and Jane, 2000; Li et al., 2001a; Yoshimoto et al., 2002; You and Izydorczyk, 2002). There is conflicting information in the literature with respect to barley amylopectin structure due to different methods [(HPSEC-MALL-RI (You and Izydorczyk, 2002), MALDI-MS (Li et al., 2001a), HPAEC-ENZ-PAD (Song and Jane,

Table 2.3 Molecular characteristics of amylose from maize and barley starches [1]

Property	Maize			Barley		
	Waxy	Regular	High amylose	Waxy	Regular	High amylose
Amylose content in starch (%)	0.62	24.45	48-68	0-11.9	23.64-33.57	37.4-55.33
Blue value	-	1.35-1.39	1.32-1.39	1.35-1.42	1.33-1.63	1.36-1.43
Iodine affinity, g/100g	-	20.0-20.1	19.4-19.6	19.5-19.8	19.0-20.1	18.8-20.0
Limited viscosity, ml/g	-	183	139-147	-	240-344	-
β-amylolysis limit, %	-	81-84	75-78	77-82	76-87	70-73
Degree of polymerization	-	930-990	690-740	1560-1680	940-1570	950-1080
Chain length	-	295-335	215-255	460-510	210-530	350-450
Chain number per molecule	-	2.8-3.4	2.9-3.2	3.3-3.4	1.8-5.3	2.4-.27
Molecular weight ($\times 10^6$)	-	3.1	1.5	30.5-40.7	4.4-5.7	2.70-2.73
Radii of gyration	-	164	83	141-149	98-107	64-65

[1] Reference: Greenwood and Thomson (1959); Banks et al. (1975); Takeda et al. (1989); Morrison and Karkalas (1990); Morrison et al. (1993b); Schulman et al. (1995); Hizukuri (1996); Takeda et al. (1999); Yoshimoto et al. (2000, 2002); Li et al. (2001[a]); You and Izydorzyk (2002); Han et al. (2005); Waduge et al. (2006).

Table 2.4 Molecular characteristics of amylopectin from maize and barley starches [1]

Property	Maize			Barley		
	Waxy	Regular	High amylose	Waxy	Regular	High amylose
Amylopectin in starch (%)	99.38	73.8	32-52	88.1-100	64-74	44.67-62.6
Blue value	-	0.110	0.427-0.441	0.04-0.12	0.07-.012	0.07-0.12
Iodine affinity, g/100g	-	1.10	3.60-4.63	0.00-0.55	0.64-0.69	0.66-1.22
Limited viscosity, ml/g	-	168	-	-	155	-
β-amylolysis limit, %	-	59	61-62	53-54	55-56	56-57
Degree of polymerization	-	10200	-	5700-8700	6000-12000	6200-7500
Chain length	18.5	19.5	30-32	17.6-22.7	19.3-21.6	19.1-20.2
Chain number per molecule	5.4	5.2	-	4.4-5.7	4.6-5.4	4.2-5.3
Molecular weight ($\times 10^6$)	254	243	197	297-305	226-284	136-141
Radii of gyration	241	247	214	249-266	223-240	164-172

[1] Reference: Morrison and Karkalas (1990); Takeda et al. (1993, 1999); Salomonsson and Sundberg (1994); Schulman et al. (1995); Hizukuri (1996); Yoshimoto et al. (2000, 2002); Li et al. (2001a); Tang et al. (2001); You and Izydorzyk (2002); Han et al. (2005); Waduge et al. (2006)

2000), gel permeation-HPLC (Yoshimoto et al., 2002)] used for detection of debranched chains. Song and Jane (2000) and Suh et al. (2004) have reported that in barley starches, the proportion of DP 6-9, DP 6-12, DP 13-24, DP 25-36 and DP > 37 are in the range 3.1–5.3, 16.5–21.6, 40.9–47.5, 14.6–17.9 and 17.8–23.7%, respectively. The highest detectable DP has been shown to be in the range 67–133 (Song and Jane, 2000; Li et al., 2001a; You and Izydorczyk, 2002). You and Izydorczyk (2002) reported that the highest DP value was observed for high amylose starch followed by normal, waxy and zero amylose. However, Song and Jane (2000) reported that normal starch contained the highest DP followed by high amylose and waxy starches.

2.3.2.2 Non carbohydrate components

Proteins, lipids and phosphorus are the minor non-carbohydrate components of barley and maize starch isolates. These components have been shown to influence hydration rates, thermal properties, retrogradation characteristics, rheological characteristics, susceptibility towards α-amylolysis and processing and product qualities of starch hydrolysates. The protein content of purified barley starches ranges from 0.1–0.4% db (McDonald and Stark, 1988; Li et al., 2001a), part of which cannot be removed even with stringent starch purification methods, and is thus, considered to be an integral part of the starch granule interior (Goldner and Boyer, 1989; Li et al., 2003). Integral proteins have been shown to be present in the central and peripheral regions of the granule (Li et al., 2003). Starch lipids are present on the granule surface and interior (Morrison, 1981; Vasanthan and Hoover, 1992; Li et al., 2001a). The surface and internal lipid contents range between 0.1–0.2% and 0.3–1.7%, respectively (Li et al., 2001a). The

internal lipid (mainly lysophospholipids) content of small barley starch granules is higher than that of large granules (Vasanthan and Bhatty, 1996), and is proportional to the amylose content (Morrison et al., 1986). Phosphorus in barley starch occurs mainly in the form of phospholipids, and ranges from 9-37, 47-66, 75-106 mg/100 g in waxy, regular and high amylose barley starches, respectively (Tester, 1997a).

2.3.3 Granule morphology

Barley starch granules consist mainly of a mixture of large lenticular granules (10-30 μm) and smaller irregularly shaped (< 6 μm) granules (Li et al., 2001a) (Figure 2.7). The large granules constitute 10-20% of the total number of starch granules and 85-90% of total starch mass; the small granules constitute 80-90% by number and 10-15% by weight (MacGregor and Fincher, 1993). The particle size distribution of barley starch granules has been reported by many investigators (MacGregor et al., 1971; Morrison et al., 1986; Tang et al., 2000; Li et al., 2001a; Tang et al., 2002; You and Izydorczyk, 2002). The wide variation observed between the results of these studies is probably due to genotypic and environmental effects and to differences in starch extraction methodologies and size measurement techniques. Scanning electron microscopy (Li et al., 2004) showed the presence of pin holes on the larger granule surface of waxy (up to 0.9 μm in diameter), and a number of tiny pinholes visible on the surface of normal and rarely visible on the surface of high-amylose barley starch granules (Figure 2.7). Transmission electron microscopy (Li et al., 2004) showed the presence of internal channels only in waxy and normal starch granules (Figure 2.8). Randomly distributed

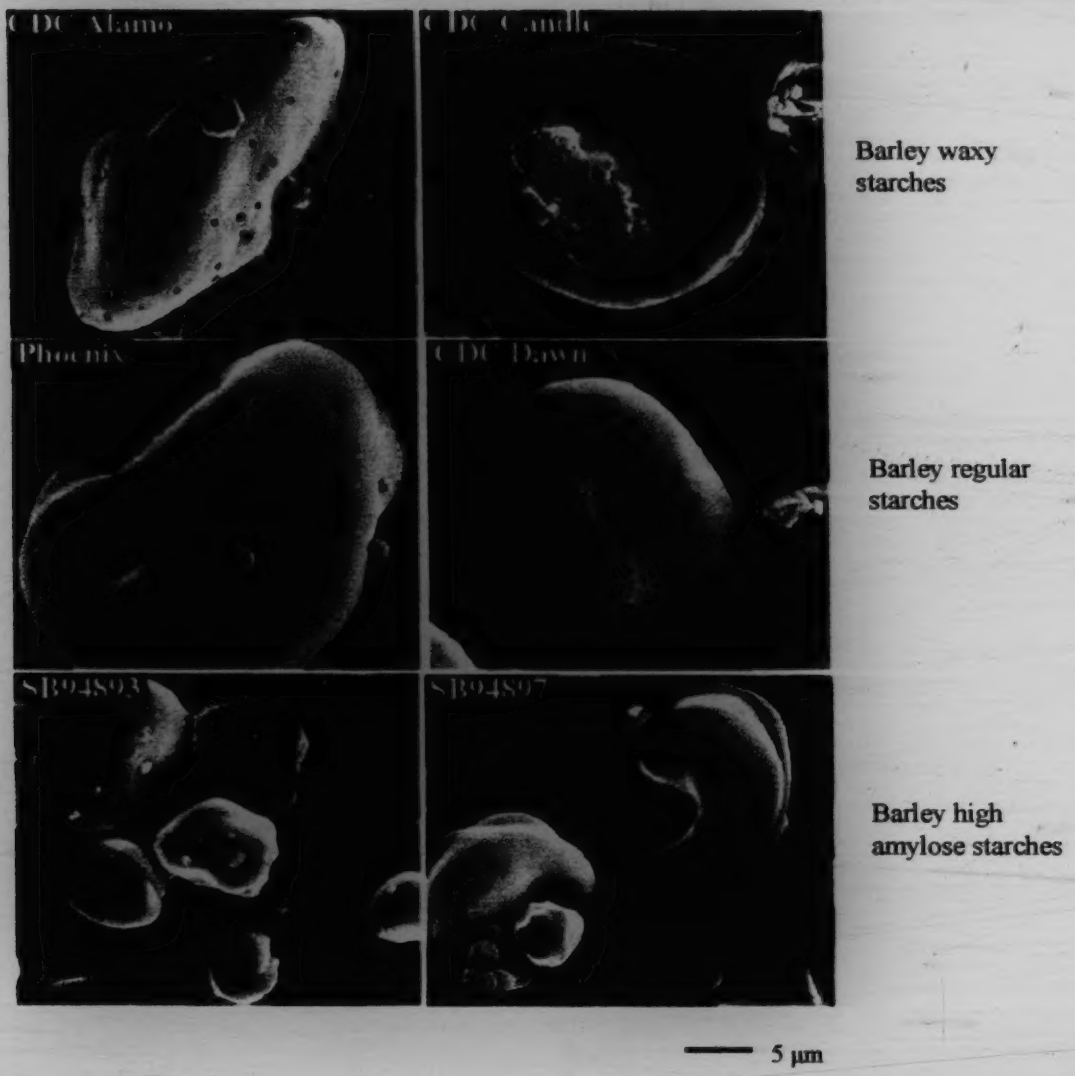

Figure 2.7 Scanning electron micrographs (SEM) of barley waxy, regular and high amylose starches (adapted from Li et al. (2001a) with minor modification).

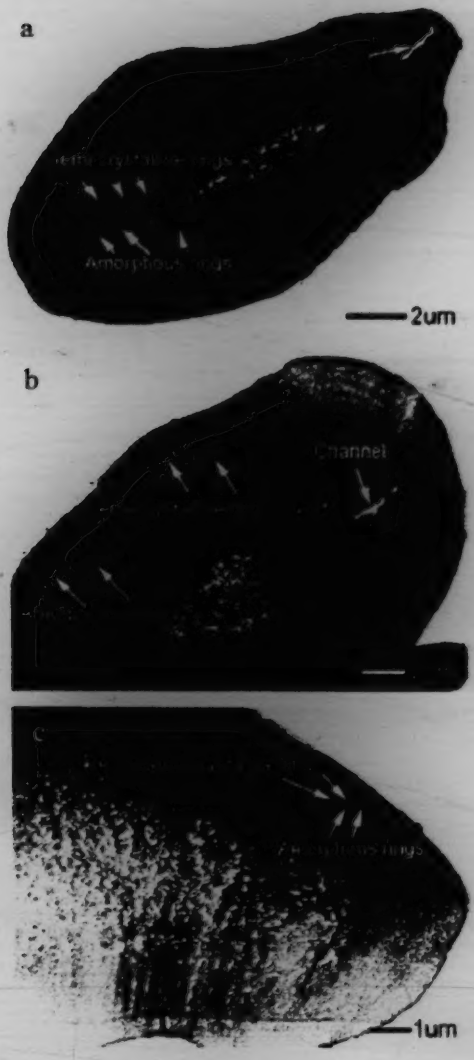

Figure 2.8 Transmission electron microscopy (TEM) of a) waxy, b) regular and c) high amylose hulless barley starch granules (adapted from Li et al. (2004) with minor modification).

pinholes have also been observed on the surface of maize, sorghum and millet starch granules (Fannon et al., 1992). These pinholes are exterior openings to internal channels that penetrate into the granule interior (Fannon et al., 2004). They, together with internal channels, are true architectural features of starch granules, potentially increasing the granule surface area available for chemical and enzymatic reactions (Huber and BeMiller, 1997, 1999, 2001; Fannon et al., 2004; Gray and BeMiller, 2004; Benmoussa et al., 2006). Maize starch granules are irregularly shaped with a number of polyhedral faces in the size of 5-20 μm and amylomaize starch granules are rod-shaped or snake-shaped (Jane et al., 1994). Atomic force microscopy (AFM) has also been used to study the starch granule surface (Ridout et al., 2004a, b).

2.3.4 X-ray diffraction and relative crystallinity

Barley and maize starches generally exhibit an A-type X-ray pattern (Figure 2.9) which is characteristic of cereal starches (Vasanthan and Bhatty, 1996; Song and Jane, 2000; Tang et al., 2001, 2002; Waduge et al., 2006). However, certain barley cultivars exhibit a mixed A + B type pattern, which is characteristic of legume starches (Waduge et al., 2006). The relative crystallinity (RC) of high amylose, regular and waxy barley starches are in the range of 20-36%, 37-42% and 33-44%, respectively (Vasanthan and Bhatty, 1996; Song and Jane, 2000; Tang et al., 2001, 2002; Waduge et al., 2006). The wide difference in relative crystallinity (RC) among regular, waxy and high amylose starches can be attributed to varietal differences and/or starch moisture content. In

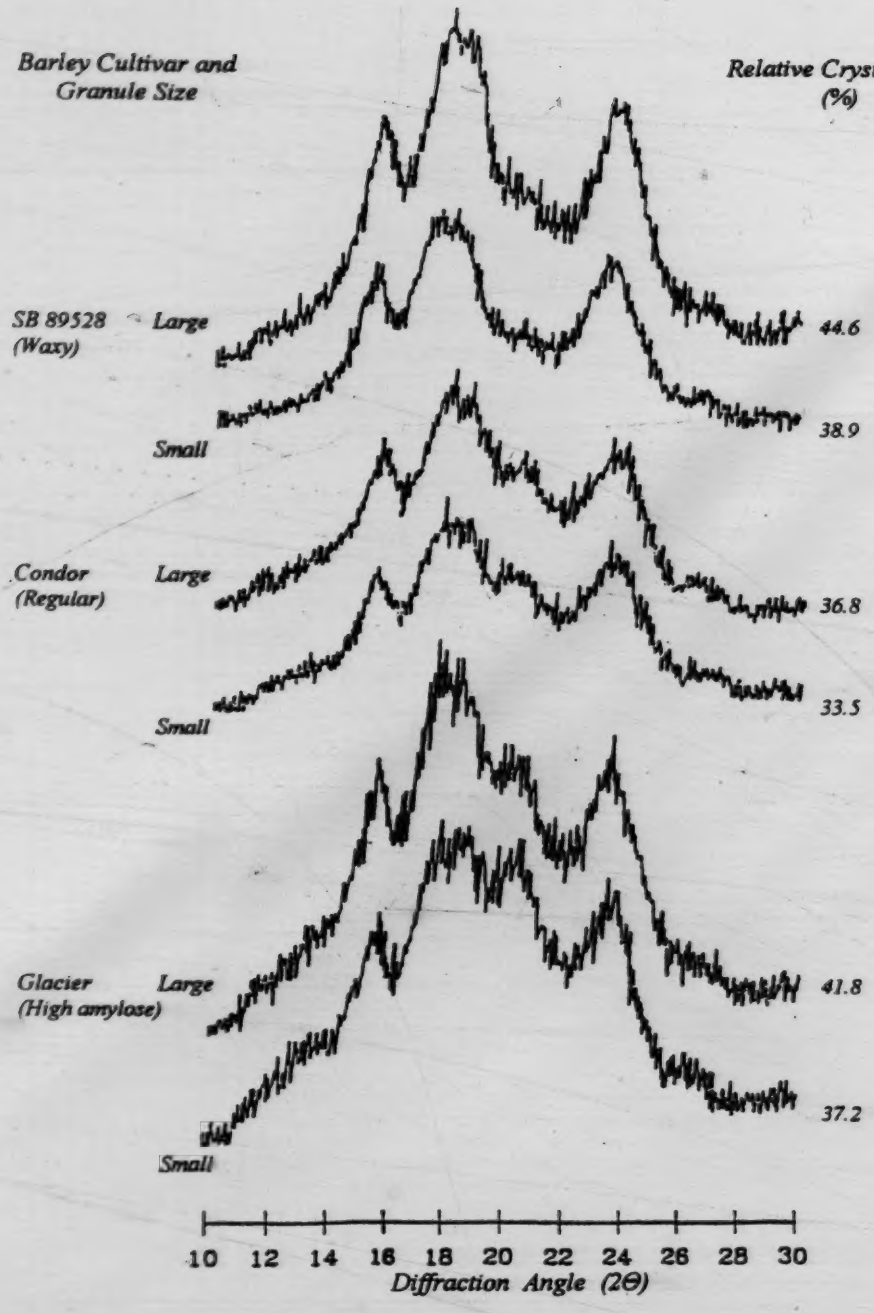

Figure 2.9 X-ray diffraction patterns and relative crystallinities of regular, waxy and high amylose starches (adapted from Vasanthan and Bhatty, (1996) with minor modification).

regular, waxy and high amylose maize starches, RC ranges 17.6-28.0%, 25-42% and 17.2-21.8%, respectively (Morsi and Sterling, 1966; Cheetham and Tao, 1998). Large granules of barley starch generally exhibit a higher RC than small granules (Figure 2.9). Irrespective of amylose content, barley starches exhibit a V-amylose-lipid complex peak centered at 20°2θ (Vasanthan and Bhatty, 1996; Waduge et al., 2006), representing crystalline V-amylose-lipid complexes (Waduge et al., 2006). However, this peak has been shown to be absent in waxy maize starch (Cheetham and Tao, 1998).

2.3.5 Gelatinization

Starch, when heated in the presence of excess water, undergoes an order-disorder phase transition called gelatinization over a temperature range characteristic of the starch source. The above phase transition is associated with the diffusion of water into the granule, water uptake by the amorphous background region, hydration and radial swelling of the starch granules, loss of optical birefringence, uptake of heat, amylose leaching and loss of molecular and crystalline order (Hoover and Hadziyev, 1981; Biliaderis, 1990; Cameron and Donald, 1992; Jenkins and Donald, 1998). The gelatinization temperature of barley starches have been studied by polarized light microscopy and differential scanning calorimetry (DSC) (MacGregor and Morgan, 1984; Kang et al., 1985; Naka et al., 1985; Tester et al., 1991; Vasanthan and Bhatty, 1996; Czuchajowska et al., 1998; Zheng et al., 1998; Li et al., 2001b; Tang et al., 2001; MacGregor et al., 2002; Yasui et al., 2002; Waduge et al., 2006). The DSC thermal characteristics of barley starch and those of its isolated granules are presented in Tables 2.5 and 2.6, respectively, and maize starches are used for comparison. The onset temperature (T_o) of gelatinization differs

Table 2.5 Gelatinization parameters of maize and barley starches [1]

Source	Type	Gelatinization Transition Temperature (°C) [2]			Enthalpy
		Onset (T_o)	Peak (T_p)	End (T_c)	(ΔH)(J/g)
Maize starch	Regular	59.8-69.2	66.9-74.1	74.9-79.5	12.3-15.5
	Waxy	60.6-71.9	67.1-76.8	74.6-83.9	13.3-15.6
	High amylose	70.6-72.8	81.6	113.8-129.4	14.5-16.2
Barley starch	Regular	52.0-61.4	58.1-65.3	62.7-74.4	11.4-14.2
	Waxy	54.5-61.3	61.8-65.5	73.8-81.8	11.4-13.1
	High amylose	53.0-61.0	62.0-68.0	74.3-76.6	6.6-12.2

[1] Reference: Jane et al. (1999); Li et al. (2001b); Qi et al. (2004); Waduge et al. (2006); Luo et al. (2007)

[2] Starch: Water 1:3

Table 2.6 Gelatinization parameters of isolated small and large granules of regular, waxy and high amylose barley starches [1]

Type	Size	Gelatinization Transition Temperature (°C) [2]			Range (°C)	Enthalpy (ΔH)
		Onset (T_o)	Peak (T_p)	End (T_c)	T_c-T_o	(J/g)
Normal	Small	54.0	61.0	70.0	16.0	6.8
	Large	54.0	58.0	67.5	13.5	7.9
Waxy	Small	60.0	64.0	76.0	16.0	9.9
	Large	59.0	62.6	72.0	13.0	11.4
High amylose	Small	58.0	68.4	79.5	21.5	6.5
	Large	59.3	65.7	77.3	18.0	7.8

[1]Reference: Vasanthan and Bhatty (1996)

[2] Starch: Water 1:3

only marginally among the barley genotypes (Table 2.5). The magnitude of the peak temperature of high amylose starch is higher than those of regular and waxy (waxy > regular). However, the conclusion temperature (T_c) is higher in waxy and high amylose (waxy > high amylose) compared to that of regular starch (Table 2.5). The enthalpy of gelatinization follows the order; regular ~ waxy > high amylose (Table 2.5). In isolated granules (Table 2.6), the difference in T_o between small and large granules of each genotype is only marginal (Table 2.6). Furthermore, small granules of each genotype exhibit higher T_p, T_c and T_c-T_o and smaller ΔH than the large granules (Table 2.6). The higher T_p, T_c and the lower ΔH of small granules have been attributed to their higher content of lipid complexed chains (Vasanthan and Bhatty, 1996). The wider T_c-T_o of small granules probably reflected the presence of crystallites of varying stability.

The wide range in T_o, T_p, T_c and ΔH among genotypes (Table 2.5), could be attributed to differences in cultivars (Tester et al., 1991; Li et al., 2001b; Waduge et al., 2006), growth conditions (Tester et al., 1991; Tester, 1997a) and thermal and mechanical conditions employed during starch isolation (Biliaderis, 1990). DSC parameters of barley starches are generally lower than those reported for regular, waxy maize starches examined under identical conditions (Li et al., 2001b).

2.3.6 Swelling factor and amylose leaching

When starch is heated in excess water, the crystalline structure is disrupted (due to breakage of hydrogen bonds) and water molecules become linked by hydrogen bonding to the exposed hydroxyl groups of amylose and amylopectin. This causes an increase in granule swelling and amylose leaching. The extent of granule swelling is determined by

measuring the swelling factor (SF), which is reported as the ratio of the volume of swollen granules to the volume of dry starch (Tester and Morrison, 1990). SF has been shown to be influenced by: 1) granule size (Vasanthan and Bhatty, 1996) 2) amylose-lipid complexes (Tester and Morrison, 1990; Vasanthan and Bhatty, 1996; Waduge et al., 2006), 3) amylopectin structure (Shi and Seib, 1992; Tester and Morrison, 1993; Sasaki and Matsuki, 1998) and 4) the extent of interaction between starch chains in the native granule (Hoover and Manuel, 1996). The SF of barley starches has been shown to follow the order: waxy > regular > high amylose (Vasanthan and Bhatty, 1996; Yashui et al., 2002; Tang et al., 2004; Waduge et al., 2006). This order is similar to that observed for maize starches (Vasanthan and Bhatty, 1996; Jayakody and Hoover, 2002). In waxy and regular barley starches, small granules exhibit a higher SF than large granules (Figure 2.10) (Vasanthan and Bhatty, 1996; Tang et al., 2002). However, in high amylose barley starch, SF is lower in the small granules (Figure 2.10). The difference in SF between the small and large granules in the barley genotypes has been attributed to the interplay of the amount of lipid complexed amylose chains and granule size (Vasanthan and Bhatty, 1996).

2.3.7 Pasting characteristics

The viscoamylography of barley starches provides information on their pasting characteristics and textural changes during cooking and cooling cycles. Marginal differences in the pasting characteristics have been reported among different barley varieties from the same type (Goering et al., 1970) and between small and large granule barley starches from the same variety (Vasanthan and Bhatty, 1996). However, greater

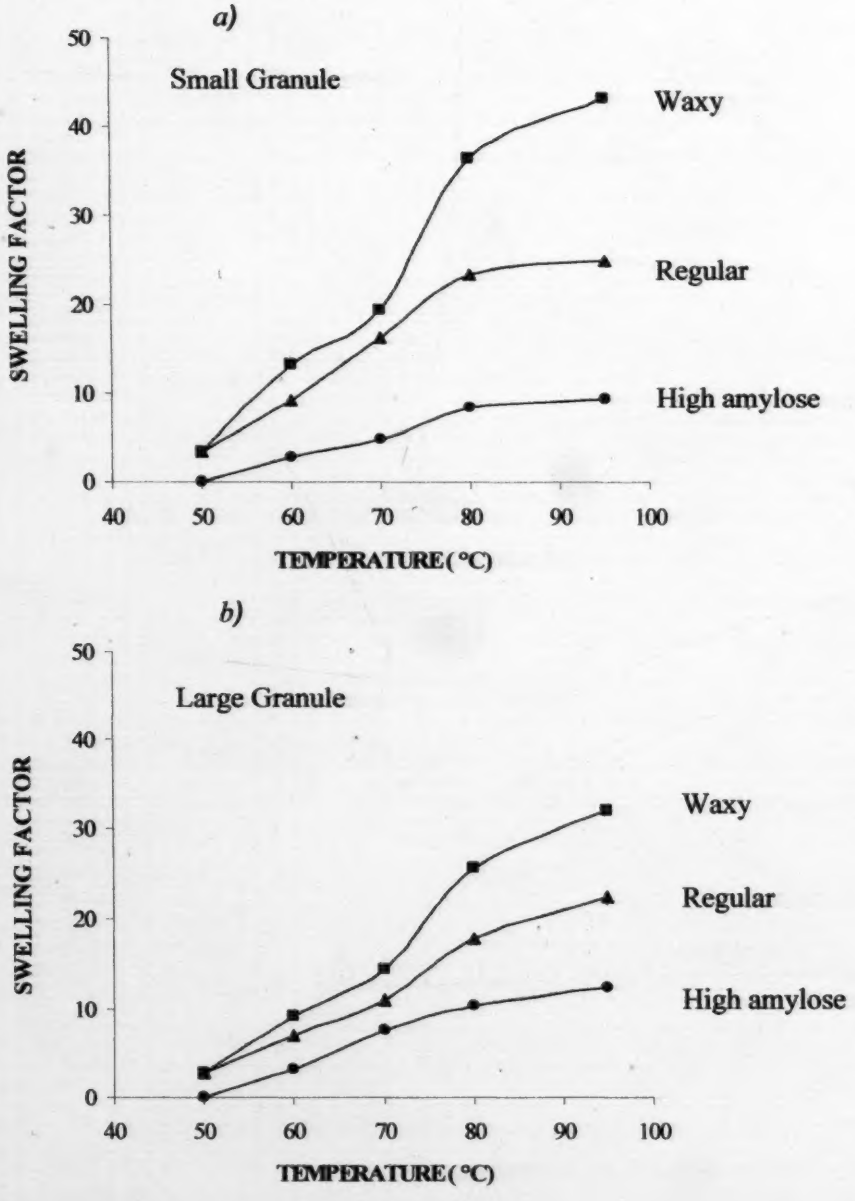

Figure 2.10 Swelling factor of small and large granules of regular, waxy and high amylose barley starches in the temperature range 50-95°C, *a*) small granules, *b*) large granules (adapted from Vasanthan and Bhatty (1996) with minor modification).

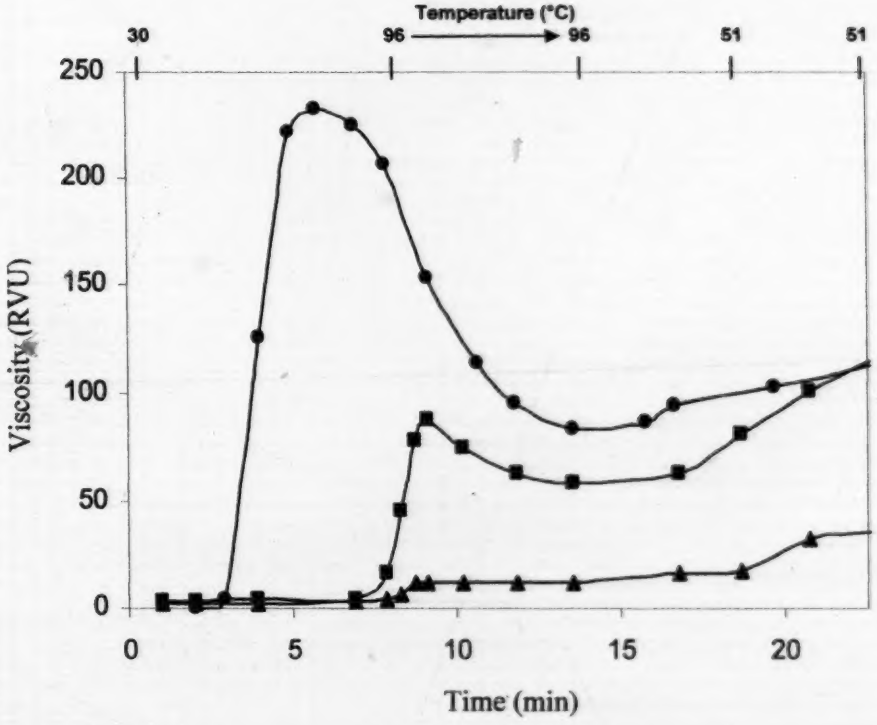

Figure 2.11 Pasting properties of barley starches measured by a Rapid Visco-Analyzer (adapted from Song and Jane (2000) with minor modification). Waxy (●), regular (■), and high amylose (▲).

differences have been reported (MacGregor and Balance, 1980; Fujita et al., 1989; Vasanthan and Bhatty, 1996; Song and Jane, 2000; Yoshimoto et al., 2002) among waxy, regular and high amylose starches (Figure 2.11) (Song and Jane, 2000). Substantially lower pasting temperature and higher peak viscosity have been recorded in waxy barley starch compared to regular or high amylose types (Figure 2.11). The thermal and shear stabilities are highest in high amylose followed by regular and waxy types (Figure 2.11). The pasting characteristics of isolated small and large granules from regular, waxy and high amylose barley starches (Vasanthan and Bhatty, 1996) are presented in Figure 2.12. Small granules of the three genotypes exhibit higher peak viscosities (Figure 2.12a) than the large granules (Figure 2.12b). In regular barley starch, set-back is higher in the large granules than in the small granules, whereas set-back is higher in the small granules of waxy and high amylose starches than in the large granules. The difference in pasting characteristics among the genotypes and between small and large granules of a particular genotype have been attributed to the interplay of differences in: 1) granule size, 2) granule swelling, 3) extent of amylose leaching, 4) V-amylose-lipid complexes, 5) phospholipid content, 6) amylose content, and 7) granule crystallinity (Takeda et al., 1989; Tester and Morrison, 1990; Shibanuma et al., 1996; Song and Jane, 2000;). The pasting curves of the barley starches (Figure 2.12) are similar to those reported for maize starches (Hoover and Manuel, 1996).

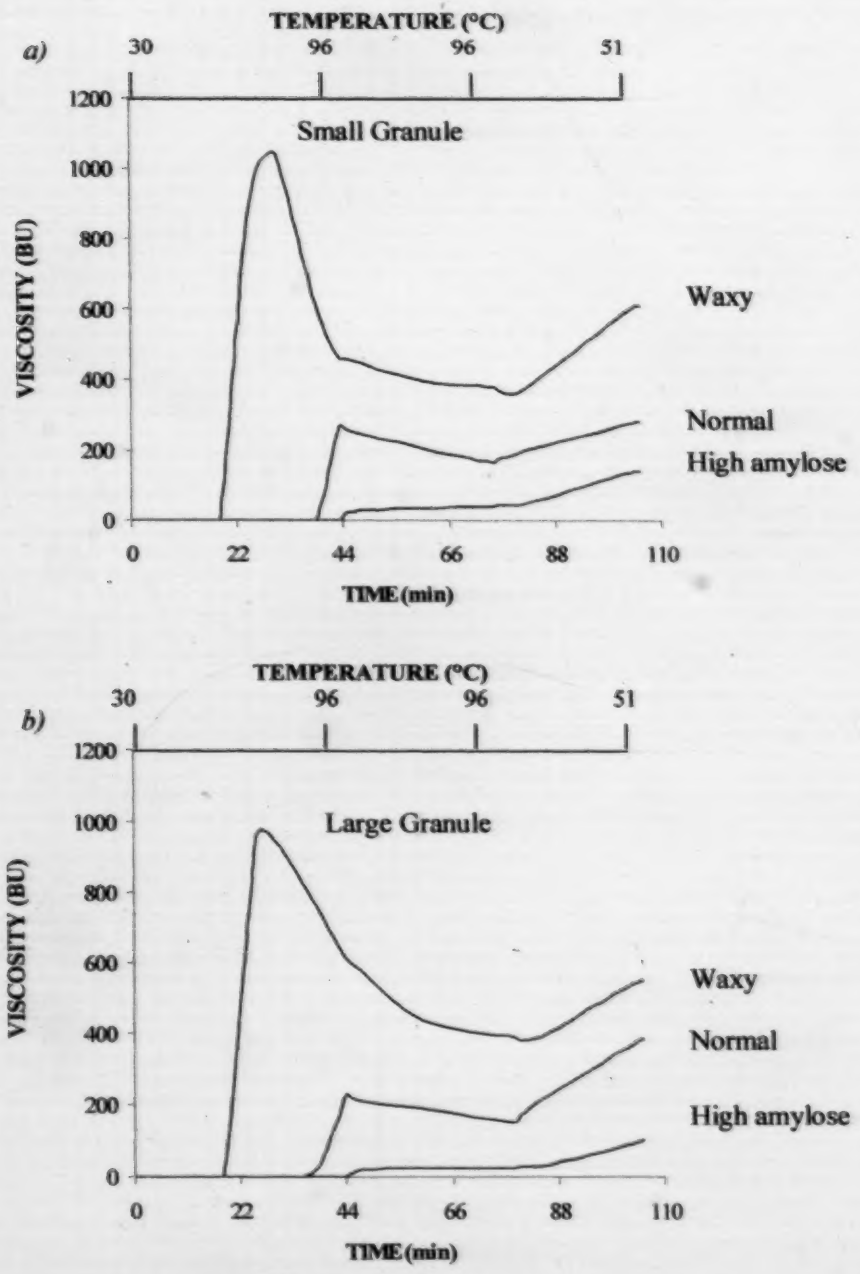

Figure 2.12 Pasting profiles of *a*) small and *b*) large granules of waxy, normal and high amylose barley starches measured by Brabender Amylography (adapted fromVasanthan and Bhatty (1996) with minor modification).

2.4 Acid hydrolysis of starch

2.4.1 Introduction

Acids cause cleavage of the glycosidic linkages, shortening the chain length, thereby altering the structure and properties of the native starch. In acid hydrolysis, H_3O^+ carries out an electrophilic attack on the oxygen atom of the α (1→4) glycosidic bond. Then the electrons in one of the carbon-oxygen bonds move onto the oxygen atom to generate an unstable, high-energy carbocation intermediate, also referred to as Lewis acid. The carbocation intermediate afterward reacts with water, leading to regeneration of a hydroxyl group and breakage of the glycosidic bond (Hoover, 2000).

2.4.2 Acid hydrolysis patterns

Starch hydrolysis by acid follows a two-stage model: a relatively fast hydrolysis stage is followed by a slow hydrolysis stage. The first stage mainly corresponds to the hydrolysis of the amorphous regions of the starch granule, whereas, the second stage corresponds to the hydrolysis of the crystalline regions within the granule (Kainuma and French, 1971; Robin et al., 1974; Biliaderis et al., 1981; Shi and Seib, 1992; Hoover and Vasanthan, 1994; Vasanthan and Bhatty, 1996; Jane et al., 1997; Jacobs et al., 1998; Gérard et al., 2002; Li et al., 2001b; Nakazawa and Wang, 2003; Waduge et al., 2006).

The extent of degradation of maize and barley starches follows the order: waxy > regular > high amylose (Li et al., 2001b; Jayakody and Hoover, 2002; Waduge et al., 2006). Among barley genotypes, small granules are hydrolyzed to a greater extent than large granules (Vasanthan and Bhatty, 1996). The difference in the extent of hydrolysis among genotypes and between small and large granules of each genotype have been

attributed to the interplay of the following factors: 1) granule size (Vasanthan and Bhatty, 1996), 2) amount of lipid complexed amylose chains (Morrison et al., 1993b), 3) double helical content (Morrison et al., 1993b), 4) extent of interaction between amylose-amylose and amylose-amylopectin chains within the native granule (Li et al., 2001a) and 5) extent of granular swelling (Song and Jane, 2000).

2.4.3 Effect of acid hydrolysis on starch granule morphology

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) are used to study starch granule morphology changes during acid treatment (Atichokudomchai et al., 2000; Jayakody and Hoover, 2002; Putaux et al., 2003; Wang et al., 2007a, b). The hydrogen ion permeates the starch granule surface then preferentially attacks the inner amorphous regions of the starch granules followed by the outer crystalline areas. Therefore, acid hydrolyzed (2.2 N HCl for 8 days) starch granules are smaller and thinner than their native counterparts and the starch granules in Chinese Yam and *Rhizoma Dioscorea* began to fracture after 16 days of hydrolysis (Wang et al., 2007a, b). Jayakody and Hoover (2002) reported that after hydrolysis with 2.2 N HCl for 8 days waxy maize starch exhibited a total loss of granular shape, followed by rice and oat starch. However, granular form was still distinct in regular maize, amylomaize V and VII starches. The surface of amylomaize V, regular and waxy maize starches were covered with numerous pores and cracks, similar to oat starch. However, pores were not shown on fragmented granules of rice starch and on the granule surface of amylomaize VII starch. Atichokudomchai et al. (2000) reported that exo-erosion all over the surface of tapioca starch granules was observed after 96 h of acid hydrolysis (6% (w/v) HCl at room temperature) and further hydrolysis caused the outer layer of the starch granule surface to

erode away. Chung and Lai (2006) reported that maize starch, hydrolyzed with 0.36% HCl in methanol at 25°C for periods of time up to 240 h, showed exo-erosion over the surface after 240 h.

2.4.4 Effect of acid hydrolysis on starch swelling

Swelling and subsequent solubilization of amylose and amylopectin are the most important structural changes during and after gelatinization of starch granules. During granule swelling, macromolecules leach out of granules resulting in a parallel increase in starch solubility. Effect of acid treatment on the swelling power of potato starch at 70°C and 80°C is shown in Figure 2.13 (Komiya and Nara, 1986). Granular swelling power at decreased 70°C and 80°C with an increase of hydrolysis time and reached the minimum value at a hydrolysis time of 6 days. When hydrolysis time increased to 8 days, swelling power increased slightly. The decrease in swelling power with hydrolysis time has also been reported by Lawal et al. (2005), Lawal (2004), and Betancur and Chel (1997). Acid preferentially breaks down the amorphous region because the crystalline area is not freely accessible to the acid and this makes it remain intact. Therefore, relative crystallinity (%) increases following acid thinning. Increase in crystallinity is responsible for the reduction in swelling capacity of the acid thinned starch, since swelling is restricted by the stiffness of entangled amylopectin networks in the crystalline region of the starch (Lawal et al., 2005).

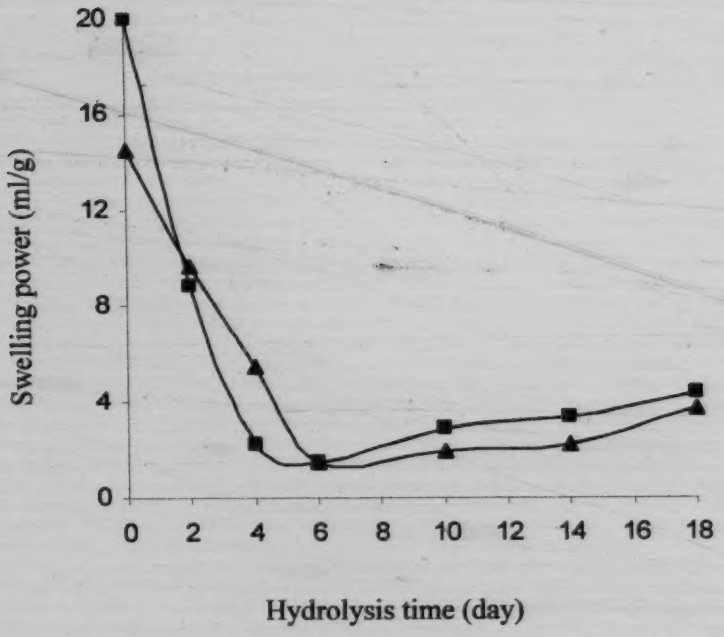

Figure 2.13 Change in swelling power at 70°C (■) and 80°C (▲) of potato starch as a function of hydrolysis time (adapted from Komiya and Nara (1986) with modification).

2.4.5 Effect of acid hydrolysis on starch crystallinity and X-ray diffraction patterns

For most starches, X-ray pattern does not change after acid hydrolysis. However, the A-type X-ray pattern of starches from certain barley cultivars and B-type X-ray pattern of cassava starch has been shown to change to a C-type pattern after acid hydrolysis (Morrison et al., 1993a; Garcia et al., 1996). Wang et al. (2007a, b) also reported that with an increase of hydrolysis time, the C-type X-ray pattern of starches from Chinese yam and Dioscoreae gradually changed to A-type X-ray pattern. The above authors have suggested that the B-type polymorph contained in the C-type polymorph mainly constituted the amorphous regions, while the crystalline areas primarily resulted from the A-type polymorph. The B-type polymorph existed in the centre of the C-type polymorph, which was surrounded by the A-type polymorph.

The degree of crystallinity of acid-hydrolyzed starches increases because acid attacks and removes the amorphous region first due to its greater accessibility within the starch granule (Muhr et al., 1984; Komiya et al., 1987; Gérard et al., 2002; Atichokudomchai et al., 2002)

2.4.6 Effect of acid hydrolysis on starch gelatinization parameters

After acid hydrolysis, the DSC endotherm of starch shifts to higher temperatures, correspondingly, the gelatinization temperature (T_o, T_p, and T_c) and the temperature range (T_c-T_o) increases in barley, maize, cassava, potato, wheat, pea, arrowroot and sweet-potato starches (Komiya and Nara, 1986; Shi and Seib, 1992; Morrison et al., 1993a; Garcia et al., 1996; Jenkins and Donald, 1997; Jacobs et al., 1998; Shi and Seib, 1998; Atichokudomchai et al., 2002; John et al., 2002; Singh et al., 2005). These phenomena

are attributed to the loss of the amorphous region of the granule where the starch residue melts at a higher temperature (Jacobs et al., 1998; Hoover, 2000).

There is no apparent trend regarding gelatinization enthalpy and acid hydrolysis, since starch source, acid concentration, hydrolysis temperature and time are variables that can affect acid hydrolysis (Biliaderis et al., 1981; Muhr et al., 1984; Komiya and Nara, 1986; Hoover and Vasanthan, 1994; Garcia et al., 1996; Jenkins and Donald, 1997; Jacobs et al., 1998; Atichokudomchai et al., 2002; John et al., 2002; Singh et al., 2005).

2.5 Starch modification

Native starches are often modified to overcome one or more of their shortcomings in functionality such as poor shear/thermal stability, undesirable texture, poor processing properties, etc., which limit the usefulness or prevent the utilization of native starches in a number of applications. Starch modifications can be classified as chemical, physical and genetic modifications (Light, 1990). Chemical modifications include conversion and derivatization. Methods of conversion include acid hydrolysis, oxidation, dextrinization, and enzyme conversion. It can increase the water solubility of starch, control gel strength, or modify the stability of starch thus allowing the use of starches at higher percentages. Methods of derivatization include crosslinking, stabilization (phosphorylation and cationization), and both crosslinking and stabilization (hydroxypropyl distarch phosphates). Crosslinking can impart resistance to processing conditions such as temperature, acid and shear. Stabilization can impart textural and freeze-thaw stability. Typical physical modifications include pregelatinization, particle size adjustment, heat-

moisture treatment, annealing and high pressure treatment. Waxy and high-amylose maize starches are products of genetic modification.

2.5.1 Starch cationization

Cationic starches are starch esters carrying a positive charge. Reagents containing amino, imino, ammonium, sulfonium, or phosphonium groups can be used to produce cationic starches. The tertiary amino and quaternary ammonium starch esters are important derivatives in the market (Solarek, 1986). They can bind with negatively charged substrates such as cellulosic fiber in papermaking industry. Cationic starches can improve sheet strength and decrease biological oxygen demand (BOD) of paper mill effluent. Presently only cationic maize and potato starches are used by Canadian paper mills. 2, 3-Epoxypropyltrimethylammonium chloride is the most popular reagent for adding quaternary ammonium groups to starch, which reacts with starch at alkaline conditions (pH 11.0-12.0). It is an epoxide that is made by reacting 3-chloro-2-hydroxypropyltrimethylammonium chloride with sodium hydroxide (Solarek, 1986) (Figure 2.14).

Cationized starch is prepared by chemical reaction of starch in a slurry or dry form in batch or continuous reactors at an alkaline pH. In the slurry system, the starch granular structure should be kept for easy recovery of starch by filtration or centrifugation; starch granule swelling and gelatiniztion should be inhibited by adding 10-30% of sodium chloride or sodium sulfate to the alkaline slurry (Carr and Bagby, 1981). Kweon et al. (1996) developed an aqueous alcoholic-alkaline process for cationizing maize and pea starches in the absence of gelatinization inhibitor salts in which

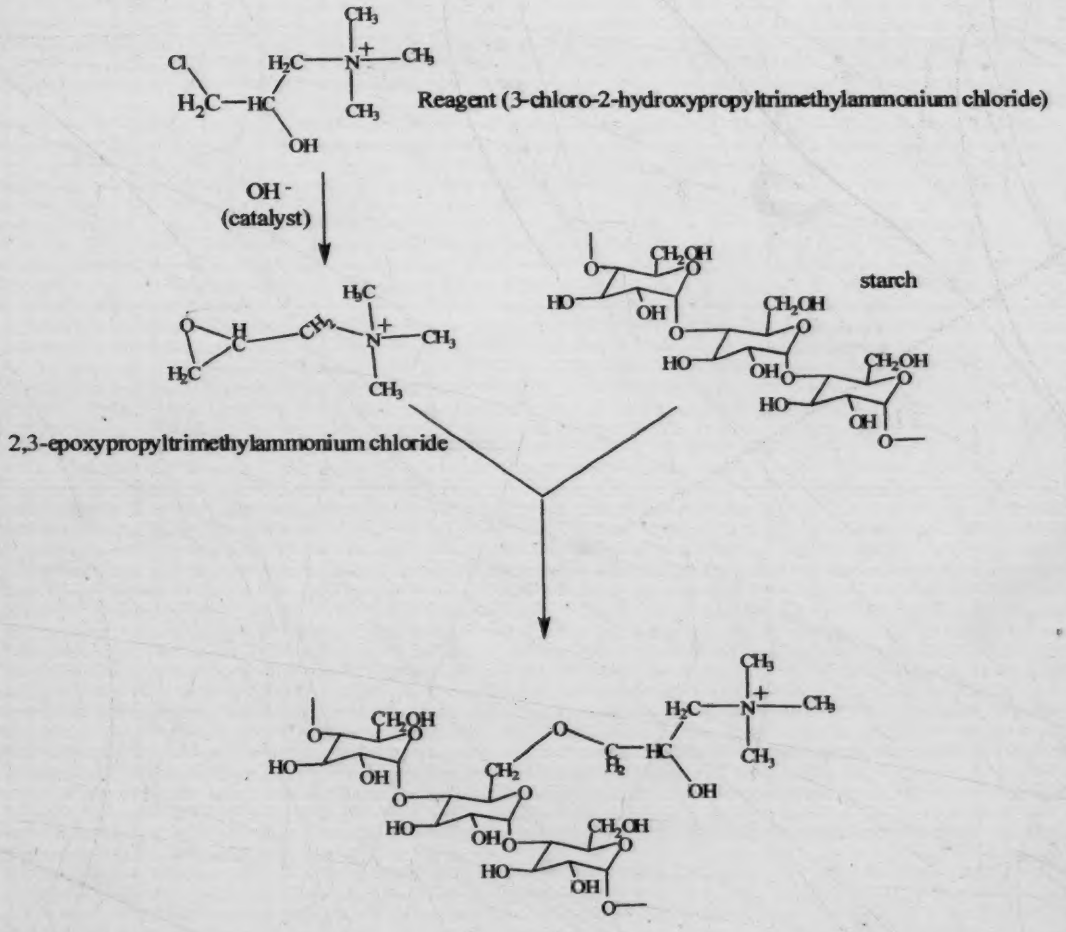

Figure 2.14 Diagram of starch cationization

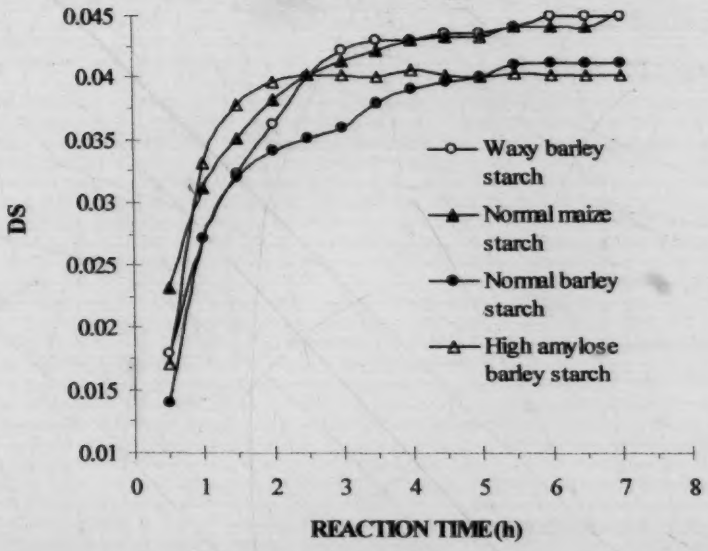

Figure 2.15 Increase in degree of substitution with reaction time during cationization of barley and normal maize starches in an alkaline-alcoholic semi-aqueous medium (adapted from Vasanthan et al. (1997) with minor modification).

the rate and efficiency of cationization were high. Vasanthan et al. (1997) applied the above technique for cationization of barley and regular maize starches. They obtained degrees of substitution (DS) of 0.041, 0.044, 0.039 and 0.044, respectively, for barley (regular, waxy, high amylose) and maize (regular) starches after a reaction time of 7 h. The average rates of reaction (DS/hour) for the above starches calculated from the initial slopes of the reaction curves (Figure 2.15) were 0.016, 0.019, 0.021 and 0.019, respectively (Vasanthan et al., 1997). Laboratory evaluation of the strength characteristics of paper sheets containing cationic barley starches were found to be superior to that of paper sheets containing laboratory modified or commercial grade (Cato-15) cationic normal maize starch, with respect to breaking length and burst index (Vasanthan et al., 1997).

A major part of the substitutions introduced by the wet-cationization method were found in the amorphous areas inside the granules and close to the branches of the amylopectin, at external chains and even spread into the crystalline lamellae. However, in the dry cationization process, the starch was substituted mainly at the surface and along the channels of the granules (Manelius et al., 2000a, b).

2.5.2 Starch phosphorylation

Starch phosphates are ester derivatives of phosphoric acid. Starch phosphates can be grouped into two classes: monostarch phosphates and distarch phosphates. Starch phosphate monoester is formed when one starch hydroxyl group is esterified to phosphate (Figure 2.16). Starch phosphate diester is formed when two starch hydroxyl groups are esterified to the same phosphate group.

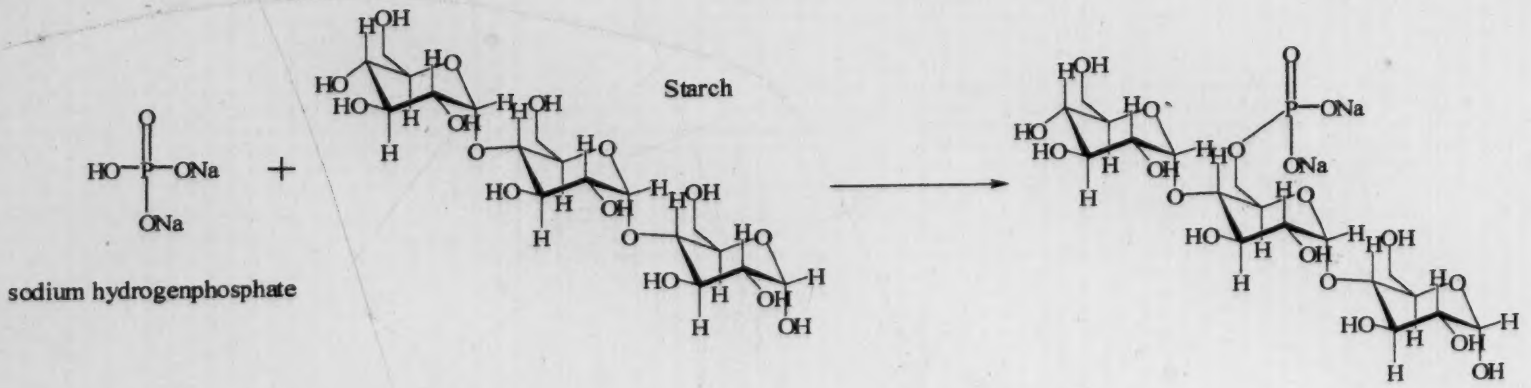

Figure 2.16 Diagram of starch phosphorylation

The monoesters of phosphoric acid is formed solely by heating a dry, intimate mixture of starch and sodium tripolyphosphate or orthophosphate salts at pH 5-6.5 (Paschall, 1962). The former only produces starch phosphates with a low degree of substitution (about 0.02 DS). However, the latter (orthophosphate salts) (mixtures of mono- and dihydrogen phosphates at pH 5-6.5) give starch phosphate with the highest degree of substitution of about 0.2 (Paschall, 1962). To avoid starch oxidation during heating, Sitohy et al. (2000a) used a vacuum oven to produce maize (0.25 DS) and rice (0.33 DS) starch phosphate monoesters. Physicochemical tests showed that a low DS (0.07) can greatly increase solubility, swelling power, and the light transmittance of modified starches; however, the above properties have been shown to decrease with an increase in DS (Sitohy et al., 2000b). Phosphorylated starches have been shown to be less susceptible to acid and α-amylase hydrolysis than their native counterparts (Sitohy and Ramadam, 2001). Negatively charged phosphate groups might preclude the hydrolytic action of α-amylase on glycosidic bonds by acting as scavenger for protons and thus buffering and easing their action (Sitohy and Ramadam, 2001). Phosphorylated starch was used to fabricate with polyacrylate, urea and water to produce thermoplastic films. The films showed both higher disintegration rate and a greater degradability by thermostable bacterial α-amylase than the ones prepared from non-phosphorylated starch. These properties are promising for producing biodegradable disposable plastic bags (Sitohy and Ramadam, 2001).

Lim and Seib (1993a) reported that phosphorylated starch prepared at pH 9.5 using a combination of 5% STPP (sodium tripolyphosphate) and 2% sodium

trimetaphosphate (STMP) in the presence of 5% sodium sulfate showed the best heat and shear stability and high paste consistency.

^{31}P-nuclear magnetic resonance (NMR) spectroscopy showed the endogenous orthophosphate groups on potato starch to be the 6- and 3-esters, and the wheat starch phosphate monoesters produced by heating starch with sodium tripolyphosphate under semidry conditions at an initial pH of 6 to contain mainly 6-monophosphate esters along with lower levels of 3- and probably 2-monophosphates (Lim and Seib, 1993b). There are no reports on barley starch phosphorylation.

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Chapter 3

An improved method for isolation of high purity starch from regular, waxy, and high amylose hulless barley grain

3.1 Introduction

Barley is a grass belonging to the family *Poaceae*, the tribe *Triticeae*, and genus *Hordeum* (Nilan and Ullrich, 1993). As the fourth most important cereal crop of the world after wheat, maize and rice, barley is widely used in animal feeding, and malting and brewing. Hulless barley (HB) has been utilized in many countries. Current usage of barley in human diets is limited due to lack of acceptance by food processors and lack of consumer awareness about the health benefits of the grain (Newman and Newman, 2004). HB production in Canada was 800, 000 tons in 1998 (Bhatty, 1999). However, due to poor agronomic and economic reasons, this crop is currently produced only under contract for certain applications.

Interest in HB utilization in the food industry has developed largely due to its high beta-glucan content, which is more predominant in the waxy cultivars (Bhatty, 1999). Furthermore, the recent approval of a health claim by the US Food and Drug Administration for barley products (US Department of Health and Human Services, Food and Drug Administration, 2006), has promoted commercial interest in barley food products.

Fractionation of barley grains and concentration of their primary components such as starch, beta-glucan, and protein has been documented in the literature (Vasanthan and Bhatty, 1995; Zheng and Bhatty, 1998; Li et al., 2001a; Vasanthan and Temelli, 2002).

Fractionation techniques include both dry and wet methods. Dry methods that are commonly used are dry-milling and sieving or air-classification (Vasanthan and Bhatty, 1995). Wet methods involve three approaches: aqueous (water), aqueous alkali (Palmer and Mackenzie, 1986) and aqueous alcohol enzymatic (Vasanthan and Temelli, 2002). Meal or flour produced through dry-milling of grains (i.e. particle size reduction) is usually the feed stock for dry and wet concentration techniques. The meal/flour is composed of a mixture of particulates that are simple (i.e., fragments of cell wall fiber, starch, protein, etc.) and complex (i.e., particulates containing cell wall fiber plus starch, starch plus protein, cell wall fiber plus starch plus protein, etc.) in nature. These particulates vary in size, shape, and density.

Simple vibratory sieving can be used to concentrate particulates according to their size into flour fractions enriched in starch, beta-glucan or protein. Air-classification of the meal/flour can also separate and concentrate particulates based on their size and density. Through optimization of air-classification parameters, such as flour feed rate, air flow rate, classifier wheel speed, etc., flour fractions enriched in starch, beta-glucan or protein can be produced. The major drawback of dry fractionation techniques is that they yield concentrates with a relatively low concentration of target components (i.e. starch, beta-glucan, protein, etc), which impose technical challenges in food formulations.

The aqueous and aqueous-alkali techniques for barley grain fractionation that are documented in the literature (Wang and Lynch, 1996; Fox, 1997) involve four major steps: 1) solubilization of beta-glucan and protein in water or aqueous alkali (alkali improves solubilization of beta-glucan and proteins); 2) centrifugation of the slurry to separate the solid phase (primarily starch and insoluble fiber) from the liquid phase

containing solubilized beta-glucan and proteins; 3) acid and/or heat precipitation of proteins from the liquid phase and subsequent removal of proteins from the liquid phase by centrifugation; and 4) recovery of beta-glucan from the liquid phase by alcohol precipitation or simple drum- or tray-drying. Starch recovered at step-2 requires further purification. Aqueous or aqueous-alkali techniques used for fractionation of barley flour raise a number of challenges due to excessive hydration and thickening of beta-glucan in water. Thickening of beta-glucan makes slurrying, filtration and centrifugation steps difficult, thus compromising the efficiency (purity and yield) of separation.

An aqueous alcohol (~50%, v/v, alcohol base) enzymatic technique (Vasanthan and Temelli, 2002) was recently introduced to concentrate beta-glucan from barley grains. In this technique, barley grain flour is initially mixed in aqueous ethanol (~50%, v/v) to prepare a uniform clump-free slurry and subsequently screened using a 50-60 micron sized sieve to obtain starch and fiber concentrates. Since beta-glucan does not hydrate and thicken in 50% ethanol, the aforementioned challenges during slurrying and filtration are totally eliminated. In addition, most of the beta-glucan is recovered in the fiber concentrate (retentate on the screen), leaving the crude starch concentrate (recovered by centrifugation of the filtrate through the screen) with trace amounts (<1%, w/w) of beta-glucan. This beta-glucan depleted crude starch fraction can be further processed easily using aqueous or aqueous-alkali techniques without encountering challenges due to hydration and thickening of beta-glucan.

The objective of the present research is three-fold: a) to investigate the feasibility of a fractionation technique that combines both aqueous alcohol and an aqueous process to isolate starch of high purity (> 90%, w/w) from regular, waxy, and high amylose

barley flours, while recovering concentrates of fiber (enriched in beta-glucan) and protein, b) to study the recovery and extraction efficiency of the process in relation to major components of barley grain such as starch, beta-glucan and protein and c) to characterize granule morphology, crystallinity, pasting and gelatinization properties of the isolated barley starches.

3.2 Materials and methods

3.2.1 Materials

Six hulless barley (HB) varieties [two waxy (WX), CDC Alamo and CDC Candle; two regular (RA), CDC Freedom and CDC McGwire; and two high amylose (HA), SB 94893 and SH 99250] grown in Saskatoon (2004) were obtained from the Crop Development Center (courtesy of Dr. Brian Rossnagel), University of Saskatchewan, Saskatoon, SK, Canada. The barley grains were ground using the Udy cyclone sample mill (UDY Lab Equipment and Supplies, Ft. Collins, CO, USA) equipped with a 0.5 mm screen for further processing. Commercial maize starches of waxy (Amioca, MWX), regular (Melojel, MRA) and high amylose (Hylon VII, MHA) varieties were obtained from National Starch and Chemical Company (Bridgewater, NJ, USA).

3.2.2 Starch isolation

Starch was isolated from ground barley grains (Udy mill with 0.5 mm sieve) according to the protocol in Figure 3.1 (a combination of aqueous alcohol and aqueous extraction). Ground barley flour was mixed with 50% ethanol (1: 4.5 w/v) in a beaker and gently stirred for 30 min. The slurry was sieved through a 63 μm screen. The fiber

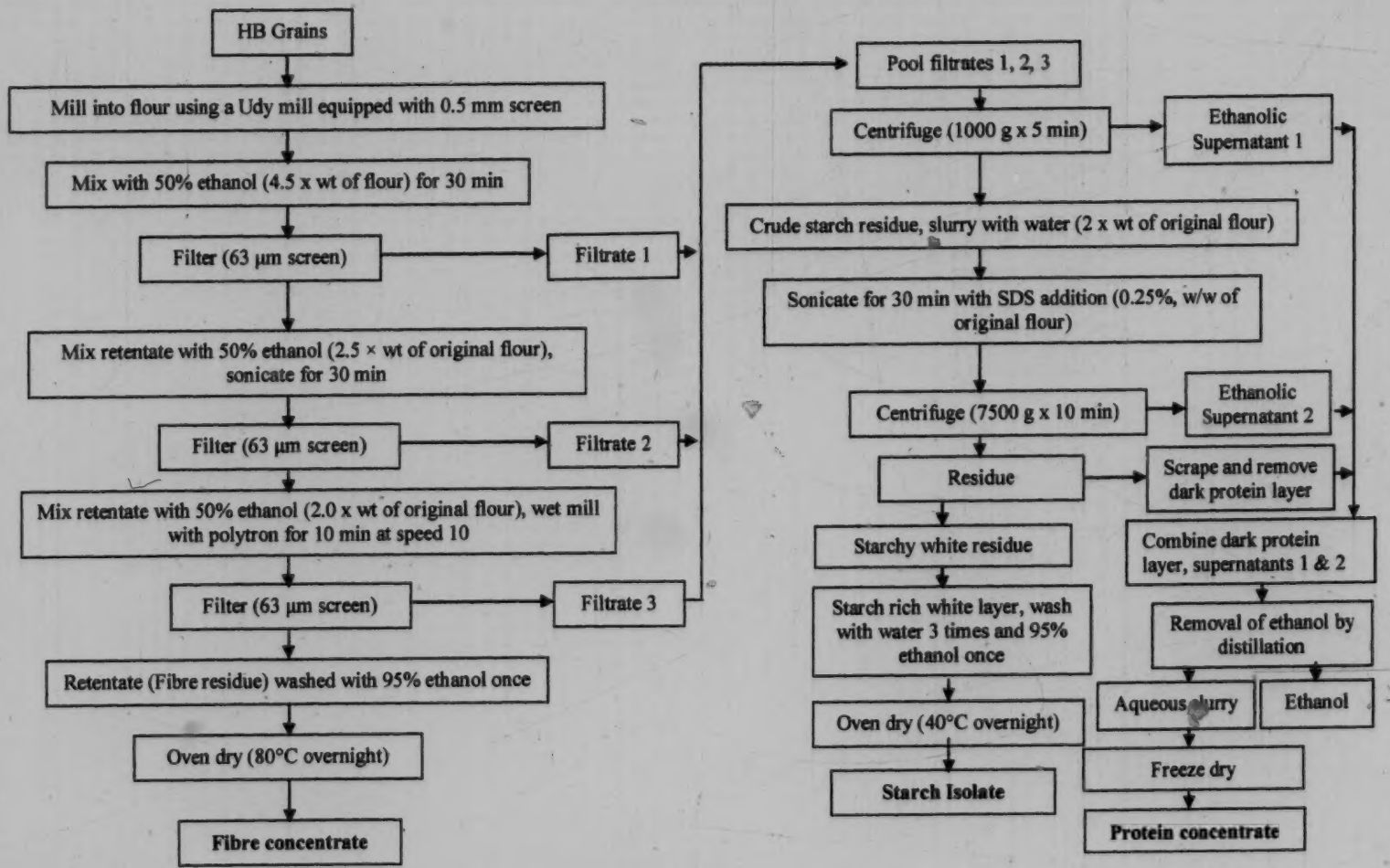
HB Grains
Mill into flour using a Udy mill equipped with 0.5 mm screen
Mix with 50% ethanol (4.5 x wt of flour) for 30 min
Filter (63 µm screen)
Filtrate 1
Mix retentate with 50% ethanol (2.5 × wt of original flour), sonicate for 30 min
Filter (63 µm screen)
Filtrate 2
Mix retentate with 50% ethanol (2.0 x wt of original flour), wet mill with polytron for 10 min at speed 10
Filter (63 µm screen)
Filtrate 3
Retentate (Fibre residue) washed with 95% ethanol once
Oven dry (80°C overnight)
Fibre concentrate
Pool filtrates 1, 2, 3
Centrifuge (1000 g x 5 min)
Ethanolic Supernatant 1
Crude starch residue, slurry with water (2 x wt of original flour)
Sonicate for 30 min with SDS addition (0.25%, w/w of original flour)
Centrifuge (7500 g x 10 min)
Ethanolic Supernatant 2
Residue
Scrape and remove dark protein layer
Starchy white residue
Combine dark protein layer, supernatants 1 & 2
Starch rich white layer, wash with water 3 times and 95% ethanol once
Removal of ethanol by distillation
Oven dry (40°C overnight)
Aqueous slurry
Ethanol
Starch Isolate
Freeze dry
Protein concentrate

Figure 3.1 Procedure for laboratory isolation of barley starches

residue on the screen was re-slurried with 50% ethanol (1: 2.5 w/v) and sonicated (Sonic 300 dismembrator (90% amplitude), Systems Corporation, Farmingdale, NY, USA) for 30 min under continuous stirring. The slurry was then sieved (63 μm) again. After sieving, the fiber residue was re-slurried with 50% ethanol (1:2 w/v) and wet-milled using a polytron homogenizer (PT 2000, Kinematica AG LITTAU, Switzerland) for 10 min (30, 000 rpm). The slurry was then sieved (63 μm) once again. The fiber concentrate was obtained by washing the retentate with 95% ethanol, followed by filtration through cheese cloth and oven drying at 80°C overnight. All filtrates through the above sievings were pooled and centrifuged (Beckman J2-21 centrifuge, Beckman Coulter, Inc., CA, USA) at 1000 g for 5 min. The supernatants were collected and the crude starch residue was re-slurried with water (1:2 w/v according to the original flour weight). SDS (sodium dodecyl sulfate) (0.25%, w/w according to the original flour) (Wang and Wang, 2004) was then added to the crude starch slurry and the mixture was sonicated (while stirring) for 30 min. The slurry was centrifuged (Beckman J2-21 centrifuge, Beckman Coulter, Inc., CA, USA) at 7500 x *g* for 10 min. The supernatant and the upper gray layer of the residue containing mainly protein with small starch granules were pooled together, vacuum evaporated to remove ethanol and freeze dried to obtain protein concentrate. The lower starch-rich white layer was washed three more times with distilled water, and finally washed with 95% ethanol. The starch isolate was dried at 40°C overnight, ground, and screened through a No. 60 mesh sieve (W.S. Tyler, ON, Canada). Dried fiber and protein concentrates were milled through 0.5 mm sieve.

3.2.3 Chemical composition of HB grains and different fractions

Quantitative estimation of moisture, ash and protein were performed by standard AACC (2000) procedures. Apparent amylose content was determined by the method of Chrastil (1987). Starch and beta-glucan contents were determined using the total starch assay kit and the mixed-linkage beta-glucan assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland), respectively.

3.2.4 Scanning electron microscopy

Granule morphology of native starches was studied by scanning electron microscopy. Starch samples were mounted on circular aluminum studs with double-sided sticky tape and then coated with 12 nm of gold, examined and photographed in a JEOL (JSM 6301 FXV) scanning electron microscope (JEOL Ltd, Tokyo, Japan) at an accelerating voltage of 5 kV.

3.2.5 Brabender viscoamylography

Pasting characteristics of the starches were determined using a viscoamylograph (Viskograph-E, C.W. Brabender Instruments, Inc., South Hackensack, NJ, USA) equipped with a 700 cm/g cartridge and operating at a bowl speed of 75 rpm. The starch slurry (6 % w/v, pH 6.5) was heated from 30 to 96°C at a rate of 1.5°C/min, maintained at 96°C for 30 min, and then cooled to 51°C at 1.5°C/min.

3.2.6 X-ray diffractometry

X-ray diffractograms were obtained with a Rigaku Geigerflex 2173 vertical goniometer (Rigaku MSC, The Woodlands TX , USA) -- X-ray diffractometer connected to a data acquisition and processing station. The starch powder (≈ 10% moisture, wet basis) was scanned through the 2Θ range of 2-50°. Traces were obtained using a Co-Kα X-ray tube with a graphite monochromator and a scintillation counter operating under the following conditions: 40 kV, 30 mA, 1°/1° divergence slit/scattering slit, 0.6 mm receiving slit, and scanning rate of 1° 2Θ/min. Jade 7.1 (Materials Data, Inc., Livermore, CA, USA) was used for data interpretation. For good comparison, all data were converted from cobalt (1.78899Å) to copper (1.54059Å) by changing the radiation wavelength. Relative crystallinity was measured by the method of Nara and Komiya (1983). Quartz was used as the 100% reference crystal.

3.2.7 Differential scanning calorimetry (DSC)

Gelatinization parameters of native and acid treated starches were measured using a Perkin-Elmer Pyris 1 DSC (The Perkin-Elmer Corporation, Norwalk, CT, USA) equipped with a data acquisition and processing station. Water (15 μL) was added with a microsyringe to native starch (5 mg, db) in an aluminum DSC pan, which was then sealed, reweighed and allowed to equilibrate for 24 h at room temperature. The scanning temperature range and the heating rate were 25-150°C and 10°C/min, respectively. In all measurements, the thermogram was recorded with an empty aluminum pan as a reference.

The thermal transitions of starch were defined in terms of temperature at T_o (onset), T_p (peak) and T_c (conclusion). The symbol ΔH refers to the enthalpy associated with the transition. This enthalpy corresponds to the area enclosed by drawing a straight line between T_o and T_c and is expressed in terms of joules per unit weight of dry starch (J/g).

3.2.8 Statistical analysis

Starch isolation was performed in two replicates and all measurements were carried out in duplicate. Analysis of variance of the results was performed using the General Linear Model (GLM) procedure of SAS Statistical Software version 9.1 (SAS Institute, 2006). Multiple comparisons of the means were performed by the Student-Newman-Keuhls test at $p < 0.05$ level.

3.3 Results and discussion

3.3.1 Chemical composition of HB grains

The chemical composition of six HB grain varieties is presented in Table 3.1. The content (%, w/w) of major components (i.e. starch, protein and beta-glucan) among different barley genotypes are in agreement with those reported elsewhere in the literature for registered Canadian barley cultivars (starch 57-68%; protein 12-16%; beta-glucan 5-8%) (Vasanthan and Bhatty, 1995; Bhatty, 1997; Bhatty and Rossnagel, 1998; Zheng and Bhatty, 1998; Andersson et al., 2001; Li et al., 2001a). HB grains from regular genotypes had higher starch ($p<0.05$) and lower protein ($p<0.05$) contents when compared to waxy or high amylose genotypes. Beta-glucan contents of waxy and high-amylose genotypes (>6.8%) were significantly higher ($p<0.05$) than that of regular types (~4.6%), leaving

Table 3.1 Chemical composition [1] of hulless barley grains

Genotype	Starch type [A]	Starch (%, db)	Protein (%, db)	Beta-glucan(%, db)	Ash (%, db)	Other [B] (%, db)
CDC Freedom	Regular	67.91 ± 1.17 [a]	12.26 ± 0.04 [f]	4.64 ± 0.08 [e]	1.88 ± 0.01 [c]	13.31
CDC McGwire	Regular	65.77 ± 0.56 [b]	13.12 ± 0.03 [e]	4.65 ± 0.14 [e]	1.85 ± 0.02 [c]	14.61
CDC Alamo	Waxy	58.95 ± 0.38 [d]	15.88 ± 0.03 [a]	7.28 ± 0.15 [c]	2.19 ± 0.02 [a]	15.69
CDC Candle	Waxy	63.46 ± 0.26 [c]	13.91 ± 0.08 [d]	6.79 ± 0.07 [d]	2.02 ± 0.01 [b]	13.81
SB 94893	High amylose	57.53 ± 0.12 [e]	15.55 ± 0.06 [b]	8.32 ± 0.02 [a]	2.19 ± 0.10 [a]	16.41
SH 99250	High amylose	58.08 ± 0.46 [de]	14.84 ± 0.02 [c]	8.05 ± 0.10 [b]	2.00 ± 0.03 [b]	17.03

[A] Based on amylose content

[B] A calculated value, mainly including insoluble dietary fiber components and lipids

[1] All data represent the mean ± standard deviation based on two determinations. Means within a column with different superscripts are significantly different ($p < 0.05$)

them highly suitable for the production of beta-glucan concentrates, a nutraceutical component that is reported to have multiple health benefits.

3.3.2 The yield and purity of fractionated products

The grain fractionation protocol (Figure 3.1) used in the present study involved both aqueous alcohol and aqueous methods. This addressed the challenges in barley starch purification. The process yielded three products: a) high purity starch isolate composed predominantly of large granules, b) low purity protein concentrate and c) low purity beta-glucan concentrate. The protein and beta-glucan concentrates required further refining to improve their purity.

Isolated starches from all six HB genotypes showed high purity (starch content >98%, w/w) with trace amounts of protein (<0.3%, w/w) and beta-glucan (<0.1%, w/w) (Table 3.2). The yield of starch isolates ranged from 22 to 39%, w/w, which was significantly ($p<0.05$) higher in regular (up to 39%, w/w) and lower in high-amylose (up to 25%, w/w) genotypes and Alamo (24.5%). Amongst the waxy genotypes, CDC Candle showed the higher starch yield (~35%, w/w). The yield of fiber concentrates was relatively higher (29 to 32%, w/w) in waxy and high amylose genotypes (31 to 32%), but lower ($p<0.05$) (17 to 22%, w/w) in regular type. The fiber concentrates were found to be enriched in beta-glucan, in the range 17 to 23% (w/w). Appreciable amounts of starch (25-39%, w/w) and protein (12-14%, w/w) were also found in this fraction. This fiber concentrate is suitable for the production of beta-glucan enriched food products or can be used as a raw-material for the production of higher purity beta-glucan isolates. The yield of the protein concentrate ranged from 32 to 46% (w/w). All protein concentrates had

Table 3.2 Yield (%, db) and composition (%, db) of hulless barley fractions obtained by the protocol outlined in Figure 3.1 [1]

Barley fractions	Yield *	Chemical Composition (%, w/w, db)		
		Starch	Protein	Beta-glucan
Starch isolate				
CDC Freedom	33.44 ^{b}	99.25 ± 1.60 ^{a}	0.19 ± 0.03 ^{a}	0.07 ± 0.02 ^{a}
CDC McGwire	39.21 ^{a}	99.71 ± 0.51 ^{a}	0.07 ± 0.02 ^{a}	0.01 ± 0.01 ^{b}
CDC Alamo	24.51 ^{cd}	99.81 ± 0.94 ^{a}	0.04 ± 0.10 ^{a}	0.06 ± 0.00 ^{a}
CDC Candle	35.69 ^{b}	99.39 ± 0.55 ^{a}	0.17 ± 0.13 ^{a}	0.09 ± 0.01 ^{a}
SB 94893	22.51 ^{d}	98.82 ± 0.88 ^{a}	0.30 ± 0.25 ^{a}	0.02 ± 0.00 ^{b}
SH 99250	25.60 ^{c}	98.58 ± 0.82 ^{a}	0.18 ± 0.17 ^{a}	0.02 ± 0.00 ^{b}
Fiber concentrate				
CDC Freedom	17.51 ^{e}	25.49 ± 0.48 ^{e}	13.64 ± 0.04 ^{a}	20.30 ± 0.06 ^{c}
CDC McGwire	22.24 ^{d}	32.92 ± 0.62 ^{d}	13.16 ± 0.14 ^{bc}	17.56 ± 0.01 ^{e}
CDC Alamo	30.10 ^{bc}	36.81 ± 0.28 ^{b}	13.37 ± 0.07 ^{ab}	21.72 ± 0.51 ^{b}
CDC Candle	29.29 ^{c}	39.09 ± 0.56 ^{a}	12.58 ± 0.02 ^{d}	19.54 ± 0.32 ^{d}
SB 94893	32.46 ^{a}	35.39 ± 0.19 ^{c}	12.91 ± 0.13 ^{c}	22.79 ± 0.00 ^{a}
SH 99250	31.50 ^{ab}	33.69 ± 0.70 ^{d}	11.82 ± 0.21 ^{e}	22.28 ± 0.03 ^{a}
Protein concentrate plus small granule starch				
CDC Freedom	45.86 ^{a}	52.03 ± 1.19 ^{b}	20.54 ± 0.03 ^{c}	1.51 ± 0.01 ^{c}
CDC McGwire	35.00 ^{d}	50.70 ± 0.07 ^{c}	26.73 ± 0.07 ^{d}	1.31 ± 0.04 ^{d}
CDC Alamo	42.40 ^{b}	48.41 ± 0.70 ^{d}	26.01 ± 0.19 ^{a}	1.19 ± 0.00 ^{e}
CDC Candle	32.62 ^{e}	41.64 ± 0.45 ^{e}	28.75 ± 0.07 ^{b}	1.71 ± 0.01 ^{b}
SB 94893	41.95 ^{b}	55.28 ± 0.82 ^{a}	24.05 ± 0.07 ^{e}	2.16 ± 0.11 ^{a}
SH 99250	40.05 ^{c}	52.19 ± 0.89 ^{b}	24.71 ± 0.07 ^{f}	2.16 ± 0.01 ^{a}

* Total grain dry weight basis.

[1] All data represent the mean ± standard deviation based on two replicate experiments followed by duplicate measurements. Means within a column with different superscripts are significantly different ($p < 0.05$) for each fraction.

protein contents twice that of the flour. The starch content of this fraction was relatively high (in the range 41-55%, w/w) and was mainly composed of small granules. This finding was in agreement to that reported by McDonald and Stark (1988).

3.3.3 Recovery and extraction efficiency

The recovery rates for starch, protein and beta-glucan were over 91% (Table 3.3) indicating only a minimal loss during extraction. Starch extraction efficiency ranged from 38 to 59% (w/w), being the highest ($p<0.05$) in CDC McGwire (59%) followed by CDC Candle (56%). The starch extraction efficiencies (38-59%, w/w) were substantially lower than those reported in the literature (>70%, w/w) where purely aqueous techniques have been employed for starch extraction (Tester and Morrison, 1992; Inagaki and Seib, 1992; Zheng et al., 1998; Andersson et al., 1999; Andersson et al., 2001; Li et al., 2001a). The lower starch extraction efficiency can be attributed to the loss of starch in fiber fraction and protein fraction, which contained the most parts of small granules. Furthermore, the new protocol (Figure 3.1) demands substantially lower amounts of water when compared to aqueous methods since most of the beta-glucan is recovered separately into a fiber concentrate by an alcohol slurrying and filtration step. Additionally, the starch isolate had predominantly large granules (more uniformity), whereas in most of the aqueous techniques outlined in the literature, both large and small starch granules were concentrated together, leading to a higher extraction efficiency, but decreased uniformity.

Table 3.3 Recovery and extraction efficiency of starch, protein and beta-glucan for barley flour fractionation by the protocol outlined in Figure 3.1 [1]

Genotype	Starch		Protein		Beta-glucan	
	Recovery (%) [A]	EE (%) [B]	Recovery (%) [A]	EE (%) [B]	Recovery (%) [A]	EE (%) [B]
CDC Freedom	90.58 ^{c}	48.87 ^{c}	96.83 ^{a}	79.35 ^{a}	92.04 ^{d}	76.61 ^{d}
CDC McGwire	97.56 ^{a}	59.44 ^{a}	93.82 ^{c}	76.00 ^{b}	93.93 ^{c}	83.99 ^{c}
CDC Alamo	95.11 ^{b}	41.50 ^{d}	94.85 ^{b}	73.22 ^{c}	96.94 ^{b}	89.80 ^{a}
CDC Candle	95.34 ^{b}	55.90 ^{b}	94.35 ^{bc}	71.46 ^{de}	92.98 ^{cd}	84.29 ^{c}
SB 94893	98.34 ^{a}	38.67 ^{e}	91.45 ^{d}	70.95 ^{e}	97.17 ^{ab}	86.23 ^{b}
SH 99250	97.71 ^{a}	43.45 ^{d}	92.09 ^{d}	72.42 ^{cd}	97.99 ^{a}	87.18 ^{b}

[A] Recovery of starch (%) = (Yield of starch isolate × Starch concentration of starch isolate +Yield of fiber concentrate × Starch concentration of fiber concentrate + Yield of protein concentrate × Starch concentration of protein concentrate)/Starch concentration of original flour.

Recovery of beta-glucan and protein were calculated as similar to the equation presented above.

[B] EE = Extraction efficiency; EE of starch (%) = Yields of starch isolate × Starch concentration of starch isolate /Starch concentration of original flour; EE of beta-glucan (%) = Yield of fiber concentrate × Beta-glucan concentration of fiber concentrate /Beta-glucan concentration of original flour; EE of protein (%) = Yield of protein concentrate × Protein concentration of protein concentrate /Protein concentration of original flour.

[1] All data represent the mean of two replicate experiments. Means within a column with different superscripts are significantly different ($p < 0.05$).

3.3.4 Microscopy

Isolated HB starches as evaluated by scanning electron microscopy (Figure 3.2a, b) at two magnification levels showed no starch damage and were free of cell wall or protein particulates. The starch isolates predominantly had oval to lenticular shaped large granules. The small granule starches were absent in the starch isolates from waxy HB, but visible in isolates from regular and high amylose (high-amylose > regular). All three types of maize starches showed the typical polyhedral shape and granule size uniformity. "Pin holes" were visible on the surface of waxy starches (both barley and maize). Equatorial grooves or furrows were present on large granules of HB and maize starches.

3.3.5 Chemical composition of HB starches

The purity of all isolated barley starches was higher than 98%. The low protein (0.04 - 0.3%) and ash (0.16 - 0.40%) contents are in the range similar to maize starches processed industrially (Table 3.4).

The apparent amylose content of the waxy starches were 1.27% and 4.34% for CDC Alamo (specific variety with zero percent amylose content, Li et al., 2001a) and CDC Candle, respectively. Regular varieties (CDC Freedom and CDC McGwire) contained around 24%, and high amylose varieties (SH 99250 and SB 94893) contained around 35% amylose. For comparison, the apparent amylose contents of waxy, regular and high-amylose maize starches were 1.58%, 24.60% and 69.27% amylose,

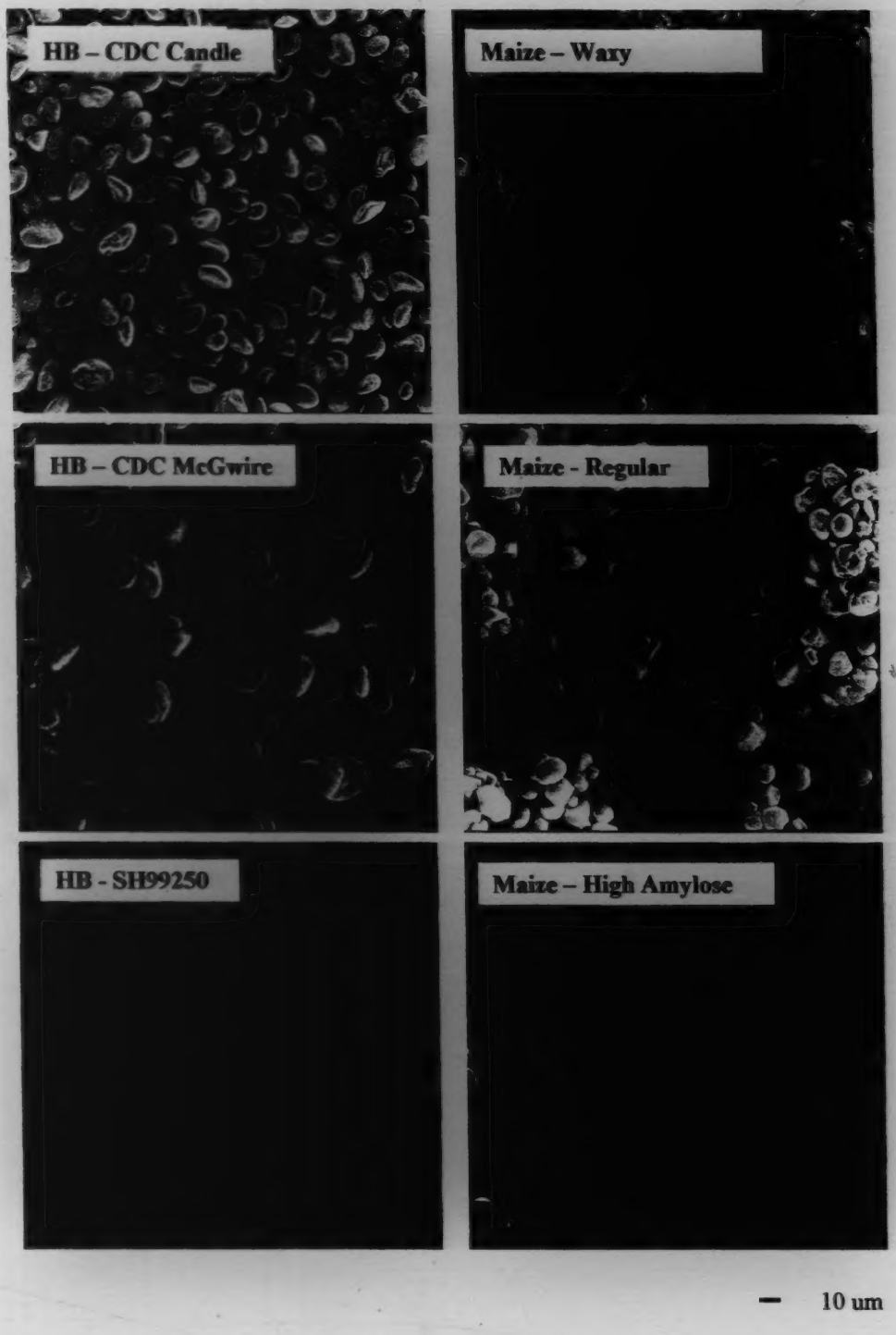

Figure 3.2a Scanning electron micrographs of purified hulless barley and commercial maize starches (magnification × 450)

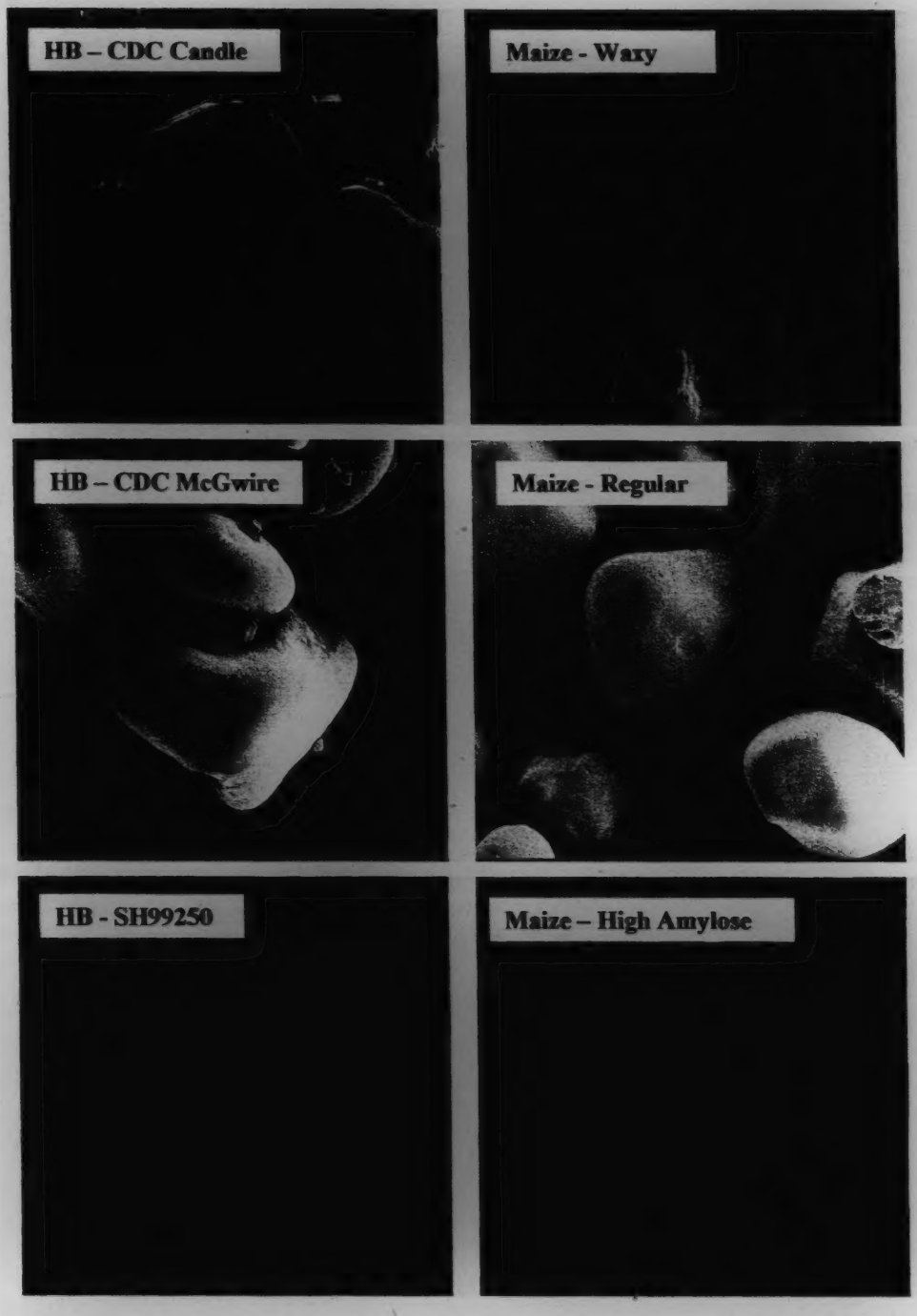

Figure 3.2b Scanning electron micrographs of purified hulless barley and commercial maize starches (magnification × 3000)

Table 3.4 Chemical composition of purified hulless barley and commercial maize starches [1]

Starch Source	Apparent Amylose (%, db)	Starch (% db)	Protein (% db)	Beta-glucan (% db)	Phosphorus (% db)	Ash (% db)
CDC Freedom	23.92 ± 2.17	99.25 ± 1.60	0.19 ± 0.03	0.07 ± 0.02	0.051 ± 0.004	0.29 ± 0.00
CDC McGwire	25.87 ± 0.39	99.71 ± 0.51	0.07 ± 0.02	0.01 ± 0.01	0.046 ± 0.001	0.30 ± 0.01
CDC Alamo	1.27 ± 0.01	99.81 ± 0.94	0.04 ± 0.10	0.06 ± 0.00	0.004 ± 0.001	0.16 ± 0.02
CDC Candle	4.34 ± 0.20	99.39 ± 0.55	0.17 ± 0.13	0.09 ± 0.01	0.011 ± 0.000	0.21 ± 0.00
SB 94893	34.14 ± 0.97	98.82 ± 0.88	0.30 ± 0.25	0.02 ± 0.00	0.075 ± 0.002	0.40 ± 0.03
SH 99250	35.37 ± 0.40	98.58 ± 0.82	0.18 ± 0.17	0.02 ± 0.00	0.055 ± 0.001	0.38 ± 0.01
Melojel	24.60 ± 0.34	99.22 ± 0.21	0.27 ± 0.20	Nd [a]	0.008 ± 0.001	0.24 ± 0.01
Amioca	1.58 ± 0.24	98.84 ± 0.09	0.23 ± 0.03	Nd	0.004 ± 0.000	0.20 ± 0.01
Hylon VII	69.27 ± 1.46	88.41 ± 0.67	0.11 ± 0.02	Nd	0.022 ± 0.000	0.24 ± 0.01

[1] All data represent the mean ± standard deviation based on two determinations.

[a] Not detected.

respectively. These above results are consistent with reported values for barley and maize starches (Vasanthan and Bhatty, 1996; Song and Jane, 2000; Yoshimoto et al., 2000; Li et al., 2001a; You and Izydorczyk, 2002; Waduge et al., 2006). The total phosphorus content including phospholipids and inorganic phosphate ranged from 0.004% to 0.075% (Table 3.4). Except for Alamo starch (0.004% total phosphates), the total phosphate contents are within the range reported by Song and Jane (2000), Tester (1997) and Waduge et al. (2006).

3.3.6 Pasting properties

The Brabender pasting curves of six HB and three maize starches are presented in Figure 3.3. In general, the pasting properties were similar to those reported in the literature (Li et al., 2001b). Waxy starches showed lower pasting temperature (58.1-63.4°C), higher peak viscosity (660 - 911 BU), and higher viscosity breakdown (478–746 BU) during the holding period than regular starches (83.3 - 89.3°C, 92-183 BU, and 15–36 BU, respectively). High amylose barley starches had a pasting temperature close to 96°C, but, a pasting temperature was not detected in HA maize starch. Peak viscosity was absent in all high amylose starches.

All HB starch isolates were mainly composed of large size granules (Figure 3.2 a, b). Vasanthan and Bhatty (1996) reported that the peak viscosity of small-granule HB waxy was higher than that of large-granule HB waxy starches. The slightly lower peak viscosity of CDC Candle and CDC Alamo, compared with other studies (Bhatty and Rossnagel, 1997; Bhatty and Rossnagel, 1998; Li et al., 2001b), may have been due to the absence of small size starch granules.

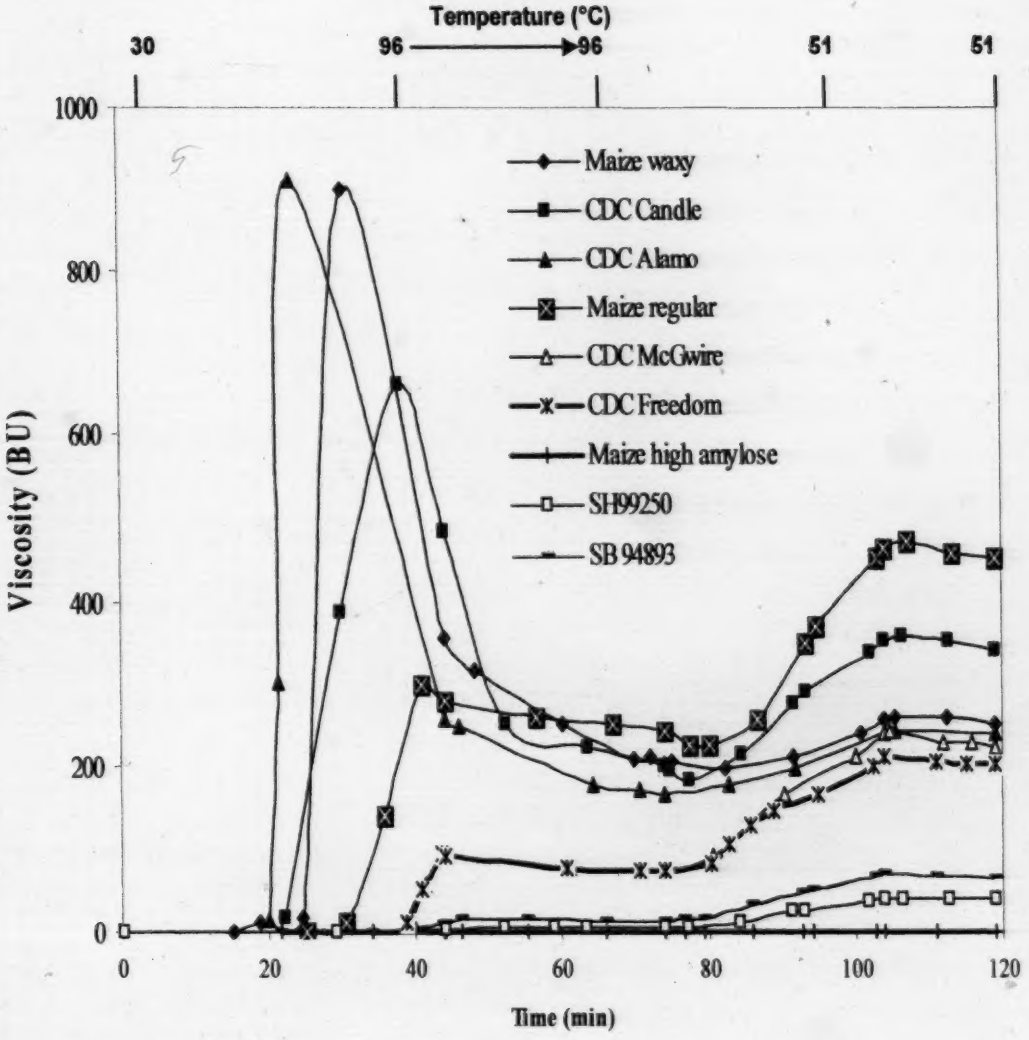

Figure 3.3 Pasting properties of hulless barley and maize starches

3.3.7 X-ray diffraction

The X-ray patterns of maize and HB starches are presented in Figure 3.4. MHA showed the typical B-type X-ray pattern with a strong peak at 2Θ 17° (d=5.2 Å), three medium peaks at 2Θ 15° (d=5.8 Å), 22° (d=4.0Å) and 24° (d=3.7Å), and a small peak at 5.5° (d=16.0 Å). All other HB and maize starches showed A-type X-ray pattern. MHA, MRA, HBHA, HBRA and HBWX also displayed a minor peak at 2Θ 20° (d = 4.5 Å) which reflected the presence of amylose–lipid complex (Zobel, 1988). RC of native MWX (41.1%), MRA (35.2%), and MHA (20.8%) are in agreement with literature values (Nara et al., 1981; Zobel, 1988). However, the results of HB starches were not in agreement with those reported by Waduge et al. (2006) for some HBHA and HBRA cultivars. The above authors reported a B-type X-ray pattern and a higher RC (39 to 42%). This discrepancy may have been due to different genotypes, differences in starch isolation techniques or growth location.

3.3.8 Differential Scanning Calorimetry (DSC)

The gelatinization transition temperatures [T_o (onset), T_p (peak), T_c (conclusion), $T_c - T_o$ (gelatinization temperature range) and ΔH (gelatinization enthalpy)] of the maize and HB starches are presented in Table 3.5. Among the maize starches, T_o, T_p, T_c and $T_c - T_o$ of MHA were significantly ($p < 0.05$) higher than those of the other two maize starches. The HB starches also showed the same trends but at a lower temperature level compared with maize starches. These results are consistent with reported values for barley and maize starches (Jane et al., 1999; Li et al., 2001b; Waduge et al., 2006; Qi et

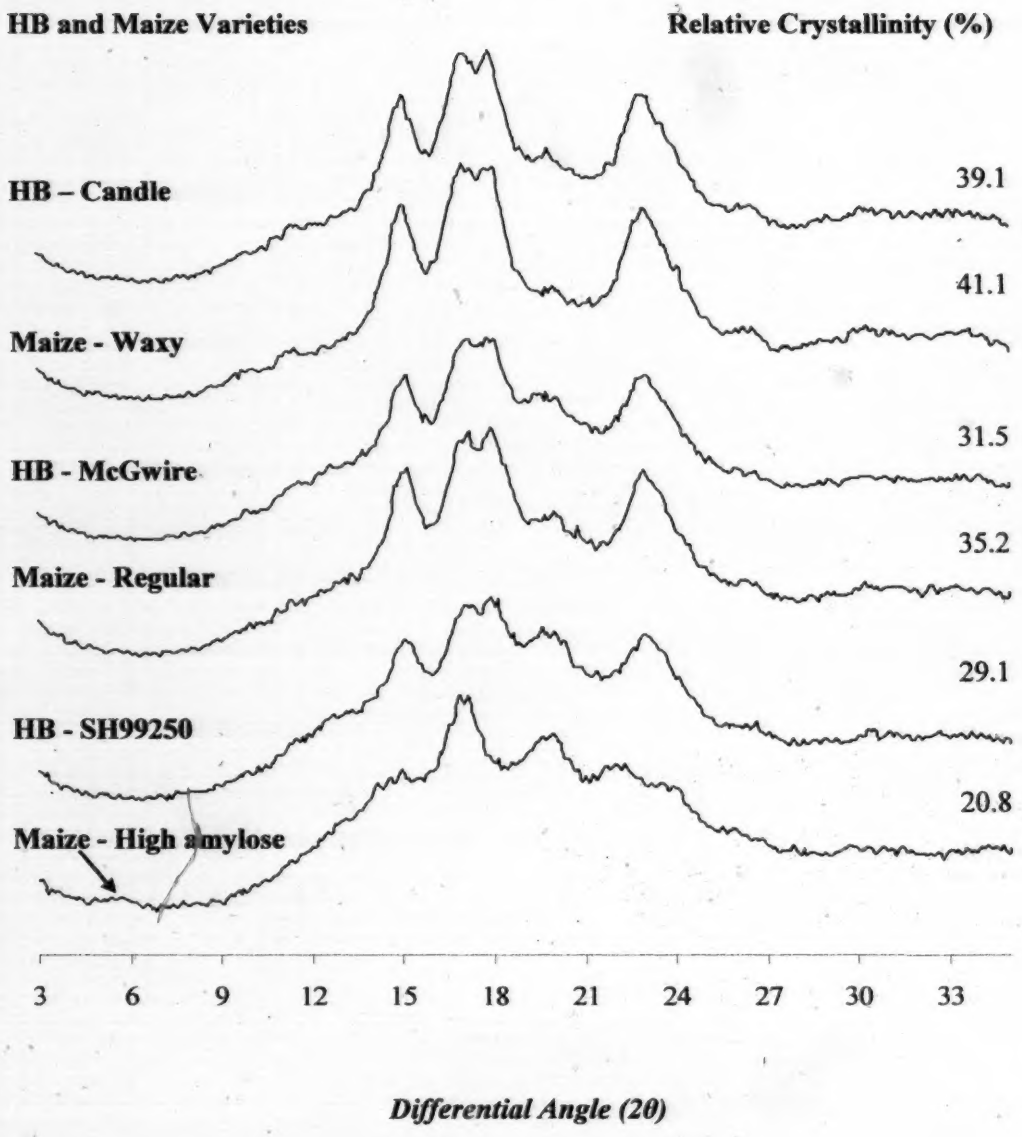

Figure 3.4 X-ray diffraction patterns and relative crystallinities of waxy, regular and high amylose starches of hulless barley and maize.The arrow points to the peak that represents the B- type unit cells.

Table 3.5 Thermal characteristics of maize and hulless barley starches [1]

Starch	Type	T_o (°C)	T_p (°C)	T_c (°C)	T_c-T_o (°C)	ΔH (J/g)
HB - McGwire	Regular	53.9 [e]	59.4 [e]	63.5 [f]	9.6 [e]	11.43 [c]
HB - Candle	Waxy	57.2 [d]	62.2 [d]	67.7 [e]	10.5 [d]	12.86 [b]
HB - SH 99250	High amylose	54.1 [e]	64.6 [c]	73.7 [d]	19.5 [b]	10.59 [c]
Maize - Melojel	Regular	66.9 [b]	71.9 [b]	76.7 [c]	10.8 [d]	13.21 [b]
Maize - Amioca	Waxy	64.9 [c]	72.5 [b]	79.2 [b]	14.3 [c]	14.26 [a]
Maize - Hylon VII	High amylose	69.2 [a]	85.0 [a]	104.6 [a]	35.4 [a]	13.00 [b]

[1] All data represent the mean of duplicate determinations. Means within a column with different superscripts are significantly different ($p < 0.05$).

al., 2004; Luo et al., 2006). The enthalpy of gelatinization followed the order: waxy > regular ≈ high amylose for both maize and HB starches.

3.4 Conclusions

Minimal technical challenges were encountered in this fractionation process and therefore the process may be suitable for large scale barley grain fractionation. All purified barley starches, isolated from crude starches (product of aqueous-alcoholic separation in the protocol) were of high purity and typical quality of their kinds. CDC Candle (waxy variety), CDC McGwire (regular variety), and SH 99250 (high amylose variety) were selected for chemical modification studies because of their high yield.

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Chapter 4

Influence of partial acid hydrolysis on the reactivity of regular, waxy and high amylose maize and barley starches towards phosphorylation and cationization

4.1 Introduction

Acid hydrolysis of starch has been the subject of a number of research investigations. The hydronium ion (H_3O^+) present in aqueous solution of acids cause cleavage of the glycosidic linkages of starch and can therefore alter its structure and properties (Hoover, 2000). The H_3O^+ carries out an electrophilic attack on the oxygen atom of the α (1→4) glycosidic bond. Then the electrons in one of the carbon-oxygen bonds move onto the oxygen atom to generate an unstable, high-energy carbocation intermediate, also referred to as Lewis acid. The carbocation intermediate subsequently reacts with water, leading to regeneration of a hydroxyl group and breakage of the glycosidic bond (Hoover, 2000). Acid preferentially attacks the amorphous regions first and then the crystalline regions of starch, giving a two-stage hydrolysis model (Kainuma and French, 1971; Robin et al., 1974; Biliaderis et al., 1981). In the native starch granule, H_3O^+ ion does not readily diffuse into the crystallites of starch granule due to tight packing of starch chains in the region. In addition, the transformation of glucose conformation from chair to half chair that is required for hydrolysis of the glycosidic bond is restricted in the crystalline region due to tight chain packing and consequent immobilization of the sugar conformation (Kainuma and French, 1971; French, 1984).

The rate and extent of acid hydrolysis among starches from different origins (i.e. cereal, tuber and legume) are primarily influenced by the extent of starch chain interactions (i.e. inter-chain associations) within the amorphous and crystalline regions of the starch granule (French, 1984; Vasanthan and Hoover, 1992). In addition, other factors such as differences in granule size (Vasanthan and Bhatty, 1996; Jane et al., 1997), extent of phosphorylation (Jane et al., 1997), lipid-complexed amylose chains (Morrison et al. 1993) and the extent of distribution of α (1→6) linkages between the amorphous and crystalline domains (Jane et al. 1997) also have shown to influence acid hydrolysis. The number and size of pores on the starch granular surface, although not investigated, can influence acid hydrolysis as well.

Barley starches, treated in 2.2 N HCl at 35°C for 18 days, exhibit a relatively high rate of acid hydrolysis during the first 10 days, followed by a slower rate (Li et al., 2001; Waduge et al., 2006). The extent of acid hydrolysis of barley and maize starches has been shown to follow the order: waxy > regular > high amylose (Li et al., 2001; Jayakody and Hoover, 2002). Extended acid hydrolysis (2.2 N HCl for 15 days at 35°C) can change starch granule morphology, X-ray crystallinity, average degree of polymerization, swelling factor, amylose leaching, gelatinization temperature range and enthalpy of gelatinization (Jayakody and Hoover, 2002). However, acid-thinned cocoyam starch (0.15 N HCl at 50°C treated for 8 h) showed an unaltered granule morphology and X-ray pattern, but decreased swelling power, increased peak temperature of gelatinization (T_p), and decreased enthalpy of gelatinization (ΔH) (Lawal, 2004). Chung and Lai (2006) reported that maize starch, hydrolyzed with 0.36% HCl (~ 0.1 N) in methanol at 25°C

and 45°C for time intervals up to 240 h, showed surface erosion after 6 h of hydrolysis at 45°C, and after 240 h at 25°C.

Acid hydrolysis can improve starch functionality. Acid hydrolyzed starches are incorporated into food formulations primarily to control viscosity, texture and moisture retention. Aqueous solutions of acid hydrolyzed starches show low viscosity and thus remain pumpable (i.e. better process control) at high concentrations (personal communication). There are also other methods of chemical modifications (i.e. cross-linking or substitution) that improve starch functionality. Starch phosphorylation and cationization are two substitution reactions that are commonly used in starch modification. Starch phosphates may be grouped into two classes: monostarch phosphates and distarch phosphates (cross-linked starches). In general, monoesters are produced with a higher level of phosphate substitution than are diesters. Gelatinized dispersions of chemically prepared starch phosphate monoesters have been reported to give clear pastes of high viscosity, with good freeze-thaw stability and emulsifying properties (Sitohy et al., 2000a). These properties allow interesting food and non-food applications of monostarch phosphates. Starch monophosphate has been traditionally prepared by slurrying dry starch in a solution containing mono- and disodium hydrogen orthophosphate, allowing the chemicals to diffuse into starch granules, recovery of starch from the slurry, mild drying followed by reacting the dry starch with a mixture of mono- and disodium hydrogen orthophosphate under high temperature (150-160°C). The actual phosphorylation reaction is triggered at high temperatures. The degree of substitution (DS) is affected by salt concentration, mixing time and reaction temperature (Sitohy et al., 2000a).

Cationic starches are widely used as wet-end additives in the pulp and paper industry to enhance starch and filler retention during paper making. Use of cationic starches increases paper strength and decreases biological oxygen demand (BOD) of paper mill effluent. Presently, cationic maize and potato starches are used by Canadian paper mills. Cationized starch is commonly prepared by a wet cationization method (Carr and Bagby, 1981). In this method, starch is suspended in aqueous alkali solution and reacted with a quarternary ammonium reagent (2,3-epoxypropyltrimethylammonium chloride). The D-glucosyl residues of starch become mono-substituted at positions C2, C3 or C6. Most of the research on starch cationization has been carried out with maize and potato starches. Limited research has been reported on the cationization of barley starches (Kweon et al., 1997a, b, c; Vasanthan et al., 1997).

Studies (Hood and Mercier, 1978; Biliaderis, 1982; Manelius et al 2000a,b) have shown that chemical substitution occurs primarily around the branch points (α 1→6) in the inter-crystalline amorphous regions of starch granules.

The hypothesis of this research was that acid hydrolysis of starch chains within the bulk and inter-crystalline amorphous regions of native granules of maize and barley starches would improve the entry of reagents used for chemical modification. The objectives were: a) to characterize the physicochemical properties of maize and barley starches from different genotypes in their native state as well as after partial (up to 240 min) acid (1 N and 2.2 N HCl) hydrolysis, and b) to study the reactivity of the hydrolyzed maize and barley starches towards phosphorylation and cationization.

4.2 Materials and methods

4.2.1 Materials

Three hulless barley (HB) varieties [CDC Candle, waxy type (HBWX); CDC McGwire, regular type (HBRA); and SH 99250, high amylose type (HBHA)] grown (year 2004) in Saskatoon were obtained from the Crop Development Center, University of Saskatchewan, Saskatoon, Canada. Commercial maize starches of waxy (Amioca, MWX), regular (Melojel, MRA) and high amylose (Hylon VII, MHA) starches were obtained from National Starch and Chemical Company (NJ, USA).

4.2.2 Starch isolation from HB grains

Isolation of starch from HB grains was carried out according to the procedure outlined in Figure 3.1.

4.2.3 Acid pre-treatment

The starches (2 g) were mixed with acid (1 N and 2.2 N) at a ratio of starch : acid (1:3, w/v) and treated in a shaking water bath (Memmert, type NB 14, Andreas Hettich GmbH, Tuttlingen, Germany) at 25°C. Aliquots (0.2 ml) were taken at specific time intervals (every 30 min up to 240 min for both 1 N and 2.2 N HCl). Control starches were produced in the same manner, but without inclusion of acid in the pre-treatment step. The filtrate obtained after acid pre-treatments at various time intervals was collected and evaluated gravimetrically for loss of hydrolyzed starch (<0.5% w/w). Aliquot (0.2 ml) of acid hydrolysate was withdrawn at specific time intervals (every 30 min up to 4h for both 1 N and 2.2 N acid), and mixed with 2.0 ml of pre-chilled 3, 5–dinitrosalicylic acid

(DNS) in plastic tubes. After adjusting the volume to 4 ml with water, tubes were chilled in ice bath for 10 min, treated in a boiling water bath for exactly 5 min and then chilled in ice bath for another 15 min. The volume of solution was then adjusted to 12 ml with water, mixed well and the absorbance was read at 540 nm. The reduction of the nitrate groups of DNS into amines and the consequent changes in the color has been the scientific basis for this determination. The appropriate standard curve of maltose was used to determine the moles of maltose equivalents in 0.2 ml aliquot and the degree of hydrolysis was correspondingly calculated (Bruner, 1964).

4.2.4 Chemical modification

Maize and barley starches were chemically modified with and without acid pre-treatment. The pre-treated starches were recovered from the slurry after neutralization by filtration and then modified chemically. There was no drying step in between acid pre-treatment and chemical modification.

4.2.4.1 Phosphorylation

Phosphorylation of starches was performed by the method of Sitohy et al. (2000a) with minor modifications. Native starch was slurried with 1 N HCl or 2.2 N HCl (1:3, w/v), treated for various time intervals, neutralized with sodium hydroxide solution (0.5 N) to pH 7.0, and filtered with suction on a fritted glass funnel. The filter cake was re-slurried in the phosphate solution [the solution contained water, sodium dihydrogen phosphate dihydrate ($NaH_2PO_4 \cdot 2H_2O$) and anhydrous disodium hydrogen phosphate (Na_2HPO_4) in the ratio of 1 : 0.304 : 0.172 (v/w/w)] in the ratio of 1:2 (w/v) (volume of

phosphate solution = 2 x original weight of starch). The slurry was stirred for 10 min and then filtered with suction on a fritted glass funnel. The filter cake was disintegrated in a coffee grinder and then dried overnight at 60°C in a forced-draft air oven. To initiate phosphorylation, the dried mixture was heated for 3 h at 160°C in a forced-draft air oven. The product was cooled and slurried in 50% aqueous methanol (1:5, w/v), stirred for 30 min, recovered on a Büchner funnel, washed three times with 50% ethanol, finally washed with 100% ethanol, and dried in a forced-draft air oven at 40-45°C. The control samples were treated with water instead of acid, filtered and taken through the same phosphorylation procedure. The bound phosphorous content (BPC) of the phosphorylated starches was determined by the method of Smith and Caruso (1964).

4.2.4.2 Cationization

Cationization of starches was performed by the method of Carr and Bagby (1981). The control and acid treated starches (the filter cake) was re-slurried in an aqueous alkali medium [water, Na_2SO_4 and NaOH in the ratio of 1:0.368:0.021, v/w/w] in the ratio of 1: 1.65 (w/v). The slurry temperature was raised to 50°C and the cationic reagent (CHPTAC—3-chloro-2-hydroxypropyltrimethyammonium chloride) was added drop-wise until an 8% (starch db, w/w) active ingredient concentration was reached in the reaction mixture. After incubation in a shaking water bath at 50°C for 5 h, the slurry was centrifuged (x1000g) and starch recovered, re-slurried in 25% ethanol (1:15 v/w), neutralized with 1 N HCl, starch recovered again by centrifugation, washed three times with 25% ethanol (by re-slurring in 25% ethanol and recovering by centrifugation), and

dried overnight at 30°C. The bound nitrogen content of the cationized starches was determined by a standard method (AACC, 2000).

4.2.5 Chemical composition of native starches

Quantitative estimation of moisture, ash and nitrogen were performed by standard AACC (2000) procedures. Apparent amylose content was determined by the method of Chrastil (1987). Phosphorus was determined by the molybdate blue method of Smith and Caruso (1964).

4.2.6 Physicochemical properties of native and acid treated starches

4.2.6.1 Granule morphology

Granule morphology of native and acid modified starches was studied by scanning electron microscopy. Starch samples were mounted on circular aluminum stubs with double-sided sticky tape and then coated with 12 nm of gold and examined and photographed in a JEOL (JSM 6301 FXV) scanning electron microscope (JEOL Ltd, Tokyo, Japan) at an accelerating voltage of 5 kV.

4.2.6.2 X-ray diffraction

X-ray diffractograms were obtained with a Rigaku Geigerflex 2173 vertical goniometer (Rigaku MSC, The Woodlands TX , USA) -- X-ray diffractometer connected to a data acquisition and processing station. The starch powder (≈ 10% moisture, wet basis) was scanned through the 2Θ range of 2-50°. Traces were obtained using a Co-Kα X-ray tube with a graphite monochromator and a scintillation counter operating under the

following conditions: 40 kV, 30 mA, 1°/1° divergence slit/scattering slit, 0.6 mm receiving slit, and scanning rate of 1° 2Θ/min. Jade 7.1 (Materials Data, Inc., Livermore, CA, USA) was used for data interpretation. For good comparison, all data were converted from cobalt (1.78899Å) to copper (1.54059Å) by changing the radiation wavelength. Relative crystallinity was measured by the method of Nara and Komiya (1983). Quartz was used as the 100% reference crystal.

4.2.6.3 Swelling factor

Swelling factor (SF) of native and acid treated starches at 80°C was measured according to the method of Tester and Morrison (1990b). The SF is reported as the ratio of the volume of swollen granules to the volume of the dry starch. Duplicate samples were used in the determination.

4.2.6.4 Differential scanning calorimetry (DSC)

Gelatinization parameters of native and acid treated starches were measured using a Perkin-Elmer Pyris 1 DSC (The Perkin-Elmer Corporation, Norwalk, CT, USA) equipped with a data acquisition and processing station. Water (15 µL) was added with a microsyringe to native starch (5 mg, db) in an aluminum DSC pan, which was then sealed, reweighed and allowed to equilibrate for 24 h at room temperature. The scanning temperature range and the heating rate were 25-150°C and 10°C/min, respectively. In all measurements, the thermogram was recorded with an empty aluminum pan as a reference.

The thermal transitions of starch were defined in terms of temperature at T_o (onset), T_p (peak) and T_c (conclusion). The symbol ΔH refers to the enthalpy associated with the transition. This enthalpy corresponds to the area enclosed by drawing a straight line between T_o and T_c and is expressed in terms of joules per unit weight of dry starch (J/g).

4.2.7 Statistical analysis

All experiments were performed in two replicates. Analysis of variance of the results was performed using the General Linear Model (GLM) procedure of SAS Statistical Software version 9.1 (SAS Institute, 2006). Multiple comparisons of the means were performed by the Student-Newman-Keuhls test at $p < 0.05$ level.

4.3 Results and Discussion

4.3.1 Acid hydrolysis of starches

The progression of acid hydrolysis (Figure 4.1 a, b) was measured at different time intervals (up to 240 min) by the change in the "reducing capacity". The reducing capacity of native starches at time zero of hydrolysis followed the order: HA > RA~WX. Each starch molecule carries a reducing end, and also since the molecular weight of amylopectin is much bigger than amylose (more than 50 times), HA are starches likely to have higher number of molecules (i.e. higher reducing capacity) as compared to starches from other genotypes. This explains the observed high reducing capacity of HA starches, and also the difference between HA maize and barley starches [the amylose content of HA maize (70%, db) is significantly higher than that of HA barley (35%, db) – Table 3.4].

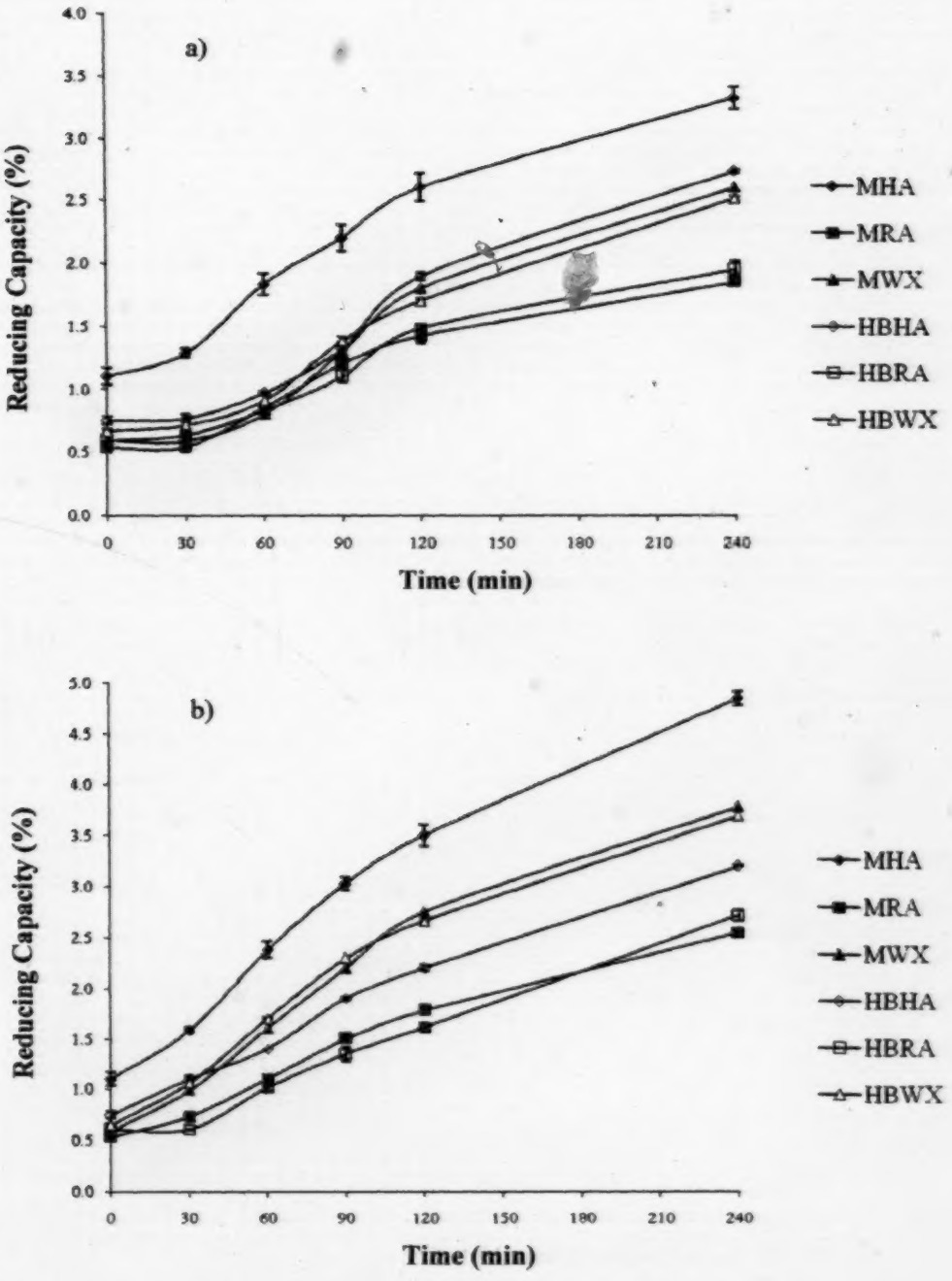

Figure 4.1 Acid hydrolysis of maize and hulless barley starches a) 1N HCl, b) 2.2 N HCl

In general, the extent of acid hydrolysis (Figure 4.1 a, b) in all starch samples at various time intervals was small (< 5%, w/w). The degree of hydrolysis (reducing capacity at time T – reducing capacity at time zero) increased with incubation time and acid strength. After 4 h of treatment, the degree of hydrolysis in maize starches at both acid concentrations (1 N and 2.2 N) followed the order: HA > WX > RA. However, in barley starches, with 1 N HCl, the degree of hydrolysis followed the same order as in maize, but for 2.2 N HCl the order changed: WX > HA> RA. The degree of hydrolysis with 2.2 N HCl was significantly ($p < 0.05$) higher (2.02-3.75% for maize and 2.12-3.04% for barley) than that obtained with 1 N HCl (1.32-2.22% for maize and 1.36-1.96% for barley). Furthermore, at both acid concentrations, maize starches (HA and WX genotypes) had significantly ($p < 0.05$) higher degree of hydrolysis than their barley counterparts, whereas there was no difference between the corresponding values for RA starches.

In the present study, the observed order in the degree of acid hydrolysis (HA > WX > RA for maize starches at both acid concentrations and for barley starches at 1 N HCl or WX > HA > RA for barley starches at 2.2 N HCl) was different from that reported for maize and barley starches (WX > RA > HA), subjected to a longer (~18 days) period of hydrolysis. For example, an extended period of acid (2.2 N HCl) hydrolysis of HB starches over 18 days at 35°C resulted in the following order: WX > RA > HA (Vasanthan and Bhatty, 1996; Li et al. 2001; and Waduge et al. 2006). A similar order was reported for maize starches (Jayakody and Hoover, 2002). The difference in the extent and rate of acid hydrolysis between starches subjected to a long period of hydrolysis have been attributed to the interplay of factors such as starch granule size

(Vasanthan and Bhatty, 1996), crystallinity (Jayakody et al., 2005), lipid complexed amylose chains (Morrison et al., 1993), amylopectin structure (Srichuwong et al., 2005), the extent of interaction between starch chains within the amorphous domains of the starch granule (Hoover and Manuel, 1996), type of unit cell (Jane, 2006), pores on the starch granule surface (Jayakody and Hoover, 2002), amylose content (Jayakody and Hoover, 2002) and amylose crystallization (Jayakody and Hoover, 2002). Amylose crystallization means that in high amylose starches, double helices in crystallites are formed to a larger extent by the intertwining of linear amylose chains, whereas in low amylose starches, double helices in crystallites are mainly formed by intertwining of the outer branches of amylopectin. Thus, double helices of high amylose starches would be more tightly packed due to strong interactions via hydrogen bonding between linear amylose chains. Such strong interactions would suppress the extent and rate of acid hydrolysis. Therefore, the interplay of the aforementioned factors must be considered when explaining the rate and extent of starch hydrolysis.

In this study, the time of hydrolysis was only 4 h, and during this time only the amorphous regions are attacked. Whereas, when hydrolysis occurs over a period of 18 days (usually the DH measurements are performed day 1 onwards), both amorphous and crystalline regions are hydrolyzed (Robin et al 1974). This could then explain the observed differences in the DH order between shorter duration (as per the present study) vs longer duration of hydrolysis. However, it is apparent based on the order of hydrolysis (Figure 4.1 a, b) that starch factors other than amylose content may become dominant in determining the extent of hydrolysis when hydrolysis time is limited to less than 240 min.

4.3.2 Starch granule surface morphology

The surface characteristics (examined by scanning electron microscopy) of maize (HA and RA) and barley starches (HA and RA) remained unchanged upon hydrolysis. However, surface pinholes were observed (Figure 4.2) in the native and acid treated maize and barley starches, which were highly pronounced in acid treated MWX. Surface pinholes in the native maize starches may have facilitated easy entry/access points for acid molecules and expansion of the pinholes (surface erosion).

4.3.3 X-ray diffraction patterns and relative crystallinity (RC) of native and acid treated starches

The X-ray patterns of native and acid treated maize and barley starches and their relative crystallinity (RC) are presented in Figure 4.3 and Table 4.1, respectively. Among the native starches, MHA showed the typical B-type X-ray pattern with a strong peak at 2Θ 17° (d=5.2 Å), three medium peaks at 2Θ 15° (d=5.8 Å), 22° (d=4.0Å) and 24° (d=3.7Å), and a small peak at 5.5° (d=16.0 Å). All other maize and HB starches showed an A-type X-ray pattern. MHA, MRA, HBHA, HBRA and HBWX also displayed a minor peak at 2Θ 20° (d=4.5 Å), which is characteristic of the amylose–lipid complex (Zobel, 1988). The RC of native maize starches from all three genotypes (20.8% for MHA, 35.2% for MRA and 41.1% for MWX) is in agreement with the values reported in the literature (Nara et al, 1981; Zobel, 1988). However, the results of HB starches did not agree with those reported by Waduge et al. (2006) for some HBHA and HBRA cultivars. The above authors reported a 'B'-type X-ray pattern and a higher RC (39 to 42%). This discrepancy may have been due to different genotypes, differences in starch isolation techniques or growth location.

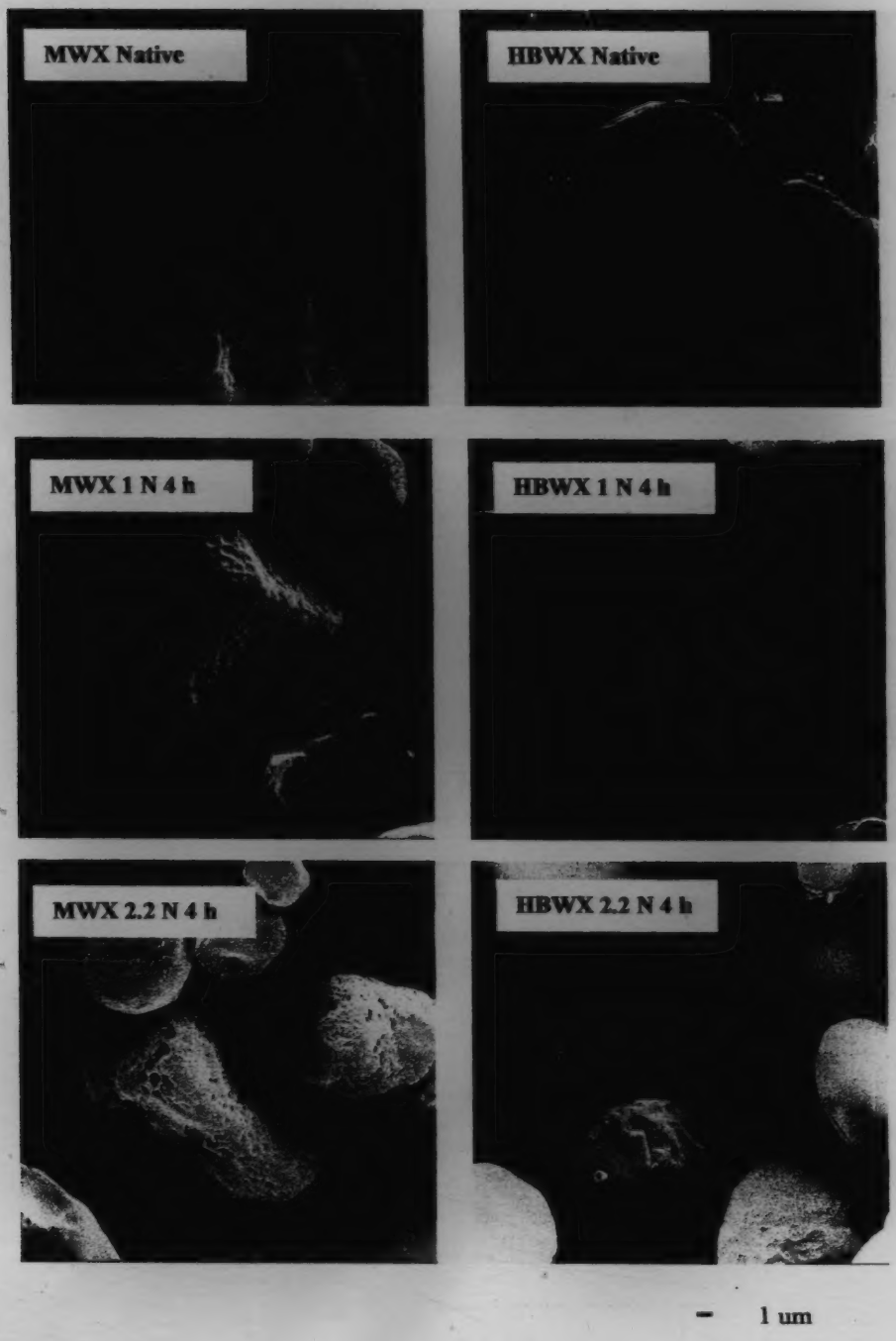

Figure 4.2 Scanning electron micrographs of MWX (maize waxy starch) and HBWX (hulless barley waxy starch) treated with HCl (magnification × 3000)

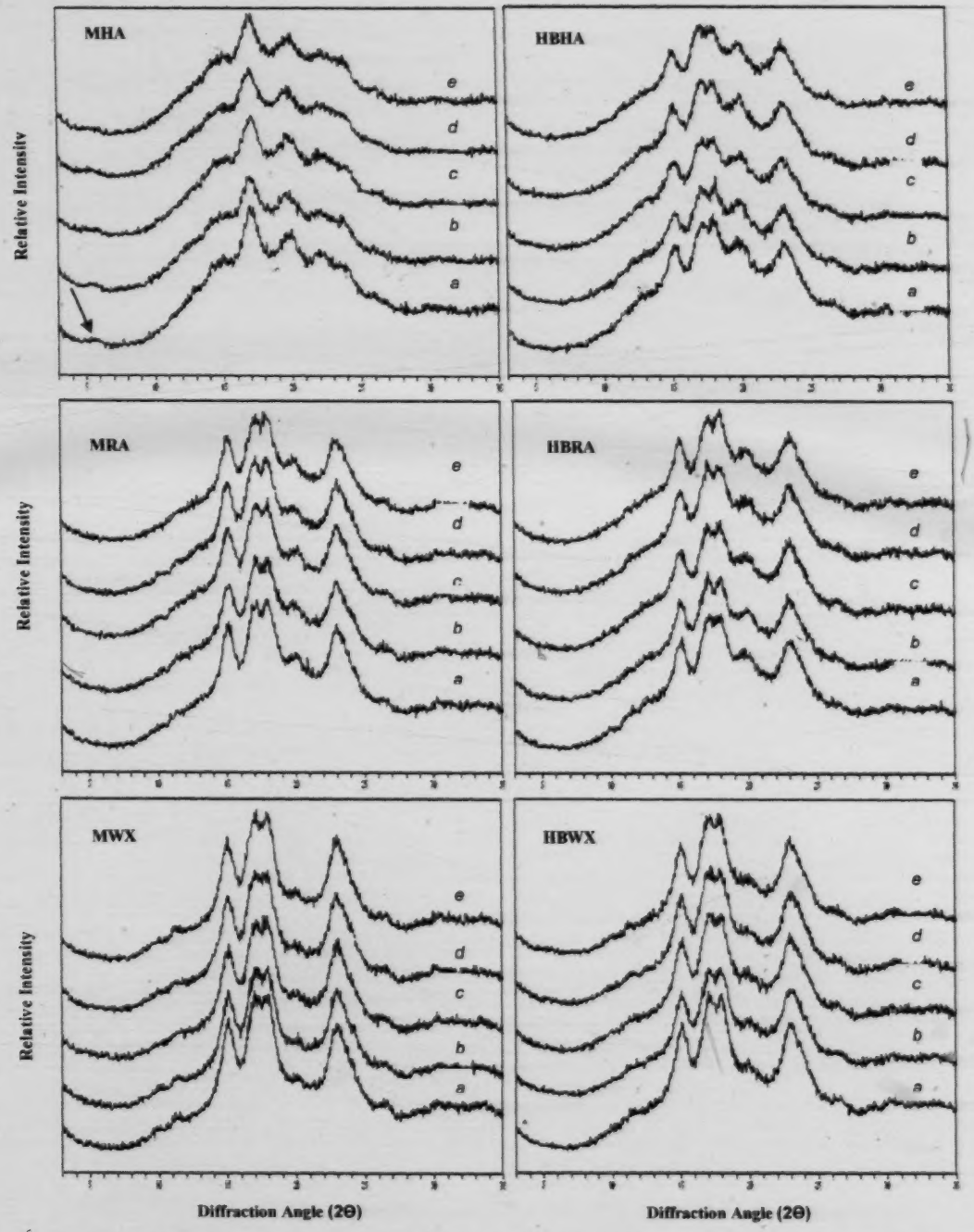

Figure 4.3 X-ray diffraction patterns of maize and barley starches, *a*) control, *b*) 1 N HCl for 90 min, *c*) 1 N HCl for 240 min, *d*) 2.2 N HCl for 90 min, and *e*) 2.2 N HCl for 240 min. The arrow points to the peak that represents the 'B' type unit cells.

Table 4.1 Relative crystallinity (%) [1] of control and acid treated maize and hulless barley starches at moisture content ~10% w/w.

	MHA	MRA	MWX	HBHA	HBRA	HBWX
Control	20.8 ^{d}	35.2 ^{bc}	41.1 ^{cd}	29.1 ^{d}	31.5 ^{bc}	39.1 ^{e}
1 N HCl						
30 min	19.9 ^{d}	35.2 ^{bc}	40.6 ^{de}	30.4 ^{c}	30.8 ^{cd}	38.6 ^{e}
90 min	22.1 ^{c}	35.4 ^{bc}	41.7 ^{c}	30.0 ^{cd}	30.7 ^{cd}	39.5 ^{de}
240 min	24.6 ^{ab}	36.7 ^{a}	43.7 ^{b}	31.9 ^{b}	32.9 ^{a}	40.3 ^{cd}
2.2 N HCl						
30 min	22.4 ^{c}	35.0 ^{c}	39.8 ^{e}	30.0 ^{cd}	30.5 ^{d}	40.6 ^{c}
90 min	23.8 ^{b}	34.4 ^{c}	43.9 ^{ab}	32.6 ^{b}	32.4 ^{ab}	42.8 ^{b}
240 min	25.3 ^{a}	36.1 ^{ab}	44.7 ^{a}	34.9 ^{a}	32.1 ^{ab}	43.9 ^{a}

[1] All data represent the mean based on two replicate experiments followed by duplicate measurements. Means within a column with different superscripts are significantly different ($p < 0.05$).

Control = starches treated in water, but without acid; MHA = maize high amylose starch; MRA = maize regular starch; MWX = maize waxy starch; HBHA = Hulless barley high amylose starch; HBRA = Hulless barley regular starch and HBWX = Hulless barley waxy starch.

Acid (1 N and 2.2 N HCl) treatment did not change the X-ray patterns (Figure 4.3) of HA, RA and WX maize and barley starches, but enhanced the RC of HA and WX maize and barley starches (Table 4.1). After 4 h hydrolysis with 2.2 N HCl, the RC increased by 21.6%, 19.9%, 8.7% and 12.2% in MHA, HBHA, MWX and HBWX starches, respectively. However during the same time period, the increase in RC with 1 N HCl was 18.2%, 9.6%, 6.3%, and 6.1%, in MHA, HBHA, MWX and HBWX starches, respectively. The RC of MRA and HBRA showed no significant ($p < 0.05$) change on all treatment except at 1 N 240 min, however, the increase in RC was smaller than that in HA and WX starches.

Srichuwong et al. (2005) and Jayakody and Hoover (2002) reported that lintnerized starches (treated with 2.2 N HCl for 15 days) retained similar X-ray patterns (i.e. crystalline type) as their native counterparts with an improvement in diffraction intensity (i.e. increase in relative crystallinity). During an extended treatment time, acid preferentially hydrolyzes and solubilizes starch chains in the amorphous regions (i.e. bulk and intercrystalline amorphous regions) (Robin et al., 1974). Therefore, during recovery of the starch residue after acid hydrolysis by centrifugation/filtration techniques, the solubilized amorphous chains are lost in the filtrate. Thus, the loss of starch granule amorphous regions and the resulting concentration of crystalline regions have been attributed to the increase in RC. However, in the present study, the loss of starch after acid hydrolysis at each time interval was carefully estimated (gravimetrically) to be negligible (<0.4% w/w). Therefore, the observed increase in RC in HA and WX starches (Table 4.1) indicates that new crystalline regions are formed. This is plausible, since acid hydrolysis of the amorphous regions of amylopectin chains

(that exist close to branch points, Figure 4.4) would facilitate the extension of double helices (Figure 4.4) therefore enhancing interhelical packing (i.e. loss of amorphous regions). Consequently, this would increase relative crystallinity (Table 4.1).

The increase in crystallinity may also be attributed to the hydrolysis of amylose chains in the bulk and intercrystalline amorphous regions. Jenkins and Donald (1995) reported that amylose can influence the arrangement of the double helices in the amylopectin crystals/unit cells by interfering with the packing density of the amylopectin chains and double helices (amylose induced disruption within the amorphous layer for packing of amylopectin chain/double helix). Similar ideas were proposed by Blanshard (1987), who reported that amylose molecules within the native starch granules exist randomly interspersed among amylopectin clusters in both "bulk amorphous" and the "intercrystalline amorphous" regions. Thus, amylose can sterically interrupt the packing of amylopectin chains/double helices. This suggests that the extent of interruption may be higher in high amylose starches. In the present study, the increase in RC is most pronounced in the high amylose starches, since hydrolysis of the amylose chains would eliminate their disruptive effect on amylopectin alignment within the crystalline lamella, resulting in improved double helical packing and enhanced crystallinity. In addition, the smaller and linear amylose chains that are generated by acid hydrolysis can efficiently retrograde via hydrogen bonding (inter chain association) to form amylose crystals (Jayakody and Hoover, 2002). Such formation of new crystals may have been responsible for the increase in RC. However, it is important to note that individual amylose chains are amorphous in nature and their hydrolysis and consequent crystal formation leads to the loss of amorphous regions.

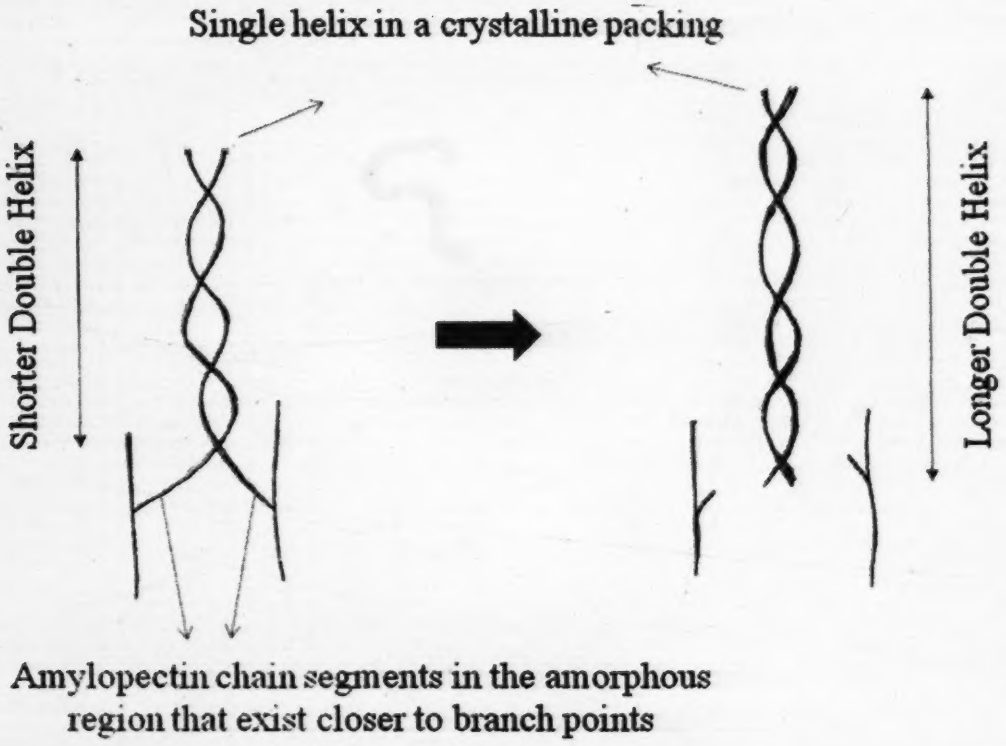
Single helix in a crystalline packing
Shorter Double Helix
Longer Double Helix
Amylopectin chain segments in the amorphous region that exist closer to branch points

Figure 4.4 Schematic illustration of a longer double helix formation due to acid hydrolysis that is responsible for the increase in relative crystallinity (RC)

In summary, it is proposed that partial acid hydrolysis of native starches can enhance new crystal formation (enhanced crystallinity) by three different mechanisms as discussed above: a) elongation/extension of the existing double helices and further packing, b) elimination of the disruptive effect of amylose on amylopectin alignment within the crystalline lamella and c) hydrolysis of amylose and formation of new crystals through retrogradation of the hydrolysed amylose chains. Based on the amylose content of the starches, it is plausible that the first proposed mechanism may be primarily responsible for the enhanced crystallinity of WX starches, since their amylose content is substantially low (<5%, db). Whereas, the enhanced crystallinity of HA starches (amylose content 35-70%, db) may be primarily due to the latter two proposed mechanisms.

Earlier researchers (Hood and Mercier, 1978; Biliaderis, 1982; Manelius et al., 2000a, b) have shown that the bulk amorphous regions of starch granules, especially the regions near the branch points of amylopectin, are the primary sites for hydration and substitution by derivatizing reagents. Therefore, it is postulated that the creation of new crystallites at the expense of amorphous regions during acid pre-treatment (resulting from the hydrolysis of both amylopectin and amylose) would compromise the hydration potential and chemical reactivity of acid treated starch granules (especially those treated at higher acid concentration and longer treatment times i.e. >120 min).

4.3.4 Swelling factor of native and acid treated starches

Swelling factor (SF) of native and acid treated starches at 80°C is presented in Table 4.2. SF is a measure of starch hydration. The SF of native starches from maize

and barley starch was the highest in the WX genotypes (82.7 for MWX and 60.6 for HBWX) followed by RA (14.5 for MRA and 11.3 for HBRA) and HA (7.0 for MHA and 7.2 for HBHA). The above order (WX > RA> HA) is in agreement with the data reported elsewhere in the literature for barley and maize starches (Vasanthan and Bhatty, 1996; Jayakody and Hoover, 2002; Waduge et al., 2006). The swelling factor of RA and WX maize starches (14.5 and 82.7, respectively) was significantly ($p<0.05$) higher than those of RA (11.3) and WX (60.6) barley starches. The HA starches from maize and barley had comparable SF values (~7) despite the substantial difference between their amylose contents (i.e. MHA, 69% and HBHA, 35%). Acid treatment significantly ($p<0.05$) decreased the SF of all maize and HB starches. The reduction in SF was inversely proportional to the time and strength of acid treatment. Amongst the genotypes, the WX starches showed the highest decrease in swelling factor. The decrease in swelling factor in WX maize and barley starches after acid treatment (2.2 N HCl) for 240 min was 69% and 46%, respectively.

Swelling is a property of amylopectin, which forms the crystalline components of the starch granules (Tester and Morrison, 1990a). However, in the present study, the observed increase in crystallinity (Table 4.1) after acid hydrolysis did not correspond to an increase in SF. In contrast, a decrease in SF was observed. Since acid initially attacks the amorphous regions of native starches, the decrease in SF upon acid treatment is indicative that amorphous regions also contribute to SF. It seems to be that in the native starch granules, the amylopectin crystalline regions that are interconnected with amorphous regions (both bulk and inter-crystalline amorphous regions) influence starch swelling. Thus, the reduced SF in the present study, may be attributed to: a) acid

Table 4.2 Swelling factor (at 80°C) of control and acid treated maize and hulless barley starches [1]

	MHA	MRA	MWX	HBHA	HBRA	HBWX
Control	7.0 ^{b}	14.5 ^{ab}	82.7 ^{a}	7.2 ^{ab}	11.3 ^{ab}	60.6 ^{a}
1 N HCl						
30 min	7.1 ^{ab}	14.7 ^{a}	81.2 ^{a}	7.0 ^{ab}	11.4 ^{ab}	54.5 ^{b}
60 min	6.8 ^{b}	14.8 ^{a}	74.8 ^{b}	6.9 ^{ab}	11.4 ^{ab}	54.5 ^{b}
90 min	7.3 ^{ab}	14.3 ^{ab}	70.6 ^{c}	6.5 ^{b}	10.5 ^{c}	50.4 ^{cd}
120 min	7.8 ^{a}	13.2 ^{c}	60.3 ^{e}	6.7 ^{ab}	10.8 ^{bc}	48.3 ^{de}
240 min	7.0 ^{b}	11.4 ^{de}	45.8 ^{g}	5.5 ^{c}	10.2 ^{cd}	43.6 ^{f}
2.2 N HCl						
30 min	6.9 ^{b}	13.7 ^{bc}	65.9 ^{d}	7.1 ^{ab}	10.2 ^{cd}	51.9 ^{bc}
60 min	7.3 ^{ab}	13.9 ^{bc}	53.2 ^{f}	6.6 ^{b}	10.3 ^{cd}	47.6 ^{de}
90 min	6.0 ^{c}	12.2 ^{d}	46.4 ^{g}	7.4 ^{a}	11.9 ^{a}	48.3 ^{de}
120 min	5.3 ^{c}	11.1 ^{ef}	39.7 ^{h}	7.3 ^{ab}	11.8 ^{a}	45.8 ^{ef}
240 min	5.5 ^{c}	10.5 ^{f}	25.4 ^{i}	5.1 ^{c}	9.50 ^{d}	32.8 ^{g}

[1] All data represent the mean based on two replicate experiments followed by duplicate measurements. Means within a column with different superscripts are significantly different ($p < 0.05$).

Control = starches treated in water, but without acid; MHA = maize high amylose starch; MRA = maize regular starch; MWX = maize waxy starch; HBHA = Hulless barley high amylose starch; HBRA = Hulless barley regular starch and HBWX = Hulless barley waxy starch.

hydrolysis of the amorphous regions and consequent disconnection between amorphous and crystalline regions of amylopectin and b) the creation of new crystalline regions (highly packed regions) at the expense of amorphous regions (as explained in the previous section) , which reduces starch hydration.

4.3.5 Thermal characteristics of native and acid treated starches

The gelatinization transition temperatures [onset (T_o), peak (T_p), conclusion (T_c)] and gelatinization enthalpy (ΔH) of control and acid treated (1N HCl) maize and HB starches are presented in Table 4.3. Sample thermograms for MWX and HBWX are presented in Figure 4.5. The gelatinization transition temperature range (T_c–T_o) for control maize and HB starches followed the order; HA > WX > RA. Whereas, the ΔH followed the order: WX > RA ~ HA. In general, the T_o, T_p, T_c and ΔH of control starches were found to be higher in maize starches than HB starches. In all starches acid hydrolysis significantly ($p < 0.05$) increased T_o and T_p, but increased T_c only marginally. T_c–T_o and ΔH decreased significantly in HA and WX starches, but remained unchanged in RA starches (Table 4.3). However, a decrease in T_o and an increase in T_p, T_c-T_o and ΔH have been observed in starches subjected to longer time periods of acid hydrolysis (Donovan and Mapes, 1980; Jacobs et al., 1998; Jayakody and Hoover, 2002).

Hydration and swelling of amorphous regions are important for starch gelatinization (Donovan and Mapes, 1980; Cooke and Gidley, 1992). Water uptake by the loosely packed amorphous regions of starch granule is accompanied by swelling within this region, which imposes a stress upon the amylopectin crystallites, resulting in the dissociation of amylopectin double helices within the crystallites. In a thermogram

Table 4.3 Thermal characteristics of control and acid treated maize and hulless barley starches [1]

Starch Source	Acid (HCl) Treatment (Acid strength and time)	Onset (T_o)°C	Peak (T_p)°C	Conclusion (T_c)°C	Range (T_c-T_o)°C	Enthalpy (ΔH) J/g
MHA	Control	69.2 ^{b}	85.0 ^{b}	104.6 ^{a}	35.4 ^{a}	13.0 ^{a}
	1 N – 30 min	70.5 ^{a}	86.7 ^{a}	104.8 ^{a}	35.2 ^{a}	12.2 ^{b}
	1 N – 90 min	No endotherm observed				
	1 N – 240 min	No endotherm observed				
MRA	Control	66.9 ^{c}	71.9 ^{b}	76.7 ^{a}	9.8 ^{a}	13.2 ^{a}
	1 N – 30 min	67.5 ^{bc}	72.2 ^{b}	77.0 ^{a}	9.5 ^{a}	13.0 ^{a}
	1 N – 90 min	68.0 ^{ab}	72.1 ^{b}	77.2 ^{a}	9.2 ^{ab}	12.9 ^{a}
	1 N – 240 min	68.6 ^{a}	73.0 ^{a}	77.3 ^{a}	8.7 ^{b}	12.7 ^{a}
MWX	Control	64.1 ^{c}	72.7 ^{c}	81.2 ^{c}	17.1 ^{a}	14.2 ^{a}
	1 N – 30 min	67.5 ^{b}	75.6 ^{b}	82.3 ^{b}	14.8 ^{b}	13.4 ^{b}
	1 N – 90 min	67.8 ^{b}	75.4 ^{b}	83.1 ^{ab}	15.3 ^{b}	13.5 ^{b}
	1 N – 240 min	69.4 ^{a}	76.9 ^{a}	83.2 ^{a}	13.8 ^{c}	12.1 ^{c}
HBHA	Control	54.1 ^{b}	64.6 ^{b}	73.7 ^{b}	19.6 ^{a}	10.6 ^{a}
	1 N – 30 min	54.5 ^{b}	64.9 ^{b}	74.0 ^{ab}	19.5 ^{a}	10.5 ^{a}
	1 N – 90 min	55.9 ^{a}	65.0 ^{b}	74.3 ^{ab}	18.4 ^{b}	10.0 ^{ab}
	1 N – 240 min	56.1 ^{a}	66.1 ^{a}	74.6 ^{a}	19.0 ^{ab}	9.20 ^{b}
HBRA	Control	53.9 ^{b}	59.4 ^{c}	63.5 ^{b}	9.6 ^{a}	11.4 ^{a}
	1 N – 30 min	53.7 ^{b}	59.9 ^{bc}	63.4 ^{b}	9.7 ^{a}	11.4 ^{a}
	1 N – 90 min	54.3 ^{b}	60.3 ^{ab}	63.8 ^{b}	9.5 ^{a}	11.1 ^{a}
	1 N – 240 min	55.8 ^{a}	60.9 ^{a}	64.9 ^{a}	9.1 ^{a}	10.9 ^{a}
HBWX	Control	57.2 ^{c}	62.5 ^{b}	68.3 ^{c}	11.1 ^{a}	12.9 ^{a}
	1 N – 30 min	60.6 ^{b}	65.9 ^{a}	69.7 ^{b}	9.1 ^{b}	11.5 ^{b}
	1 N – 90 min	61.2 ^{ab}	65.3 ^{a}	70.8 ^{a}	9.6 ^{b}	11.2 ^{b}
	1 N – 240 min	61.5 ^{a}	65.9 ^{a}	70.6 ^{a}	9.1 ^{b}	10.3 ^{c}

[1] All data represent the mean based on two replicate experiments followed by duplicate measurements. Means within a column with different superscripts are significantly different ($p< 0.05$) for each cultivar.

Control = starches treated in water, but without acid; MHA = maize high amylose starch; MRA = maize regular starch; MWX = maize waxy starch; HBHA = Hulless barley high amylose starch; HBRA = Hulless barley regular starch and HBWX = Hulless barley waxy starch.

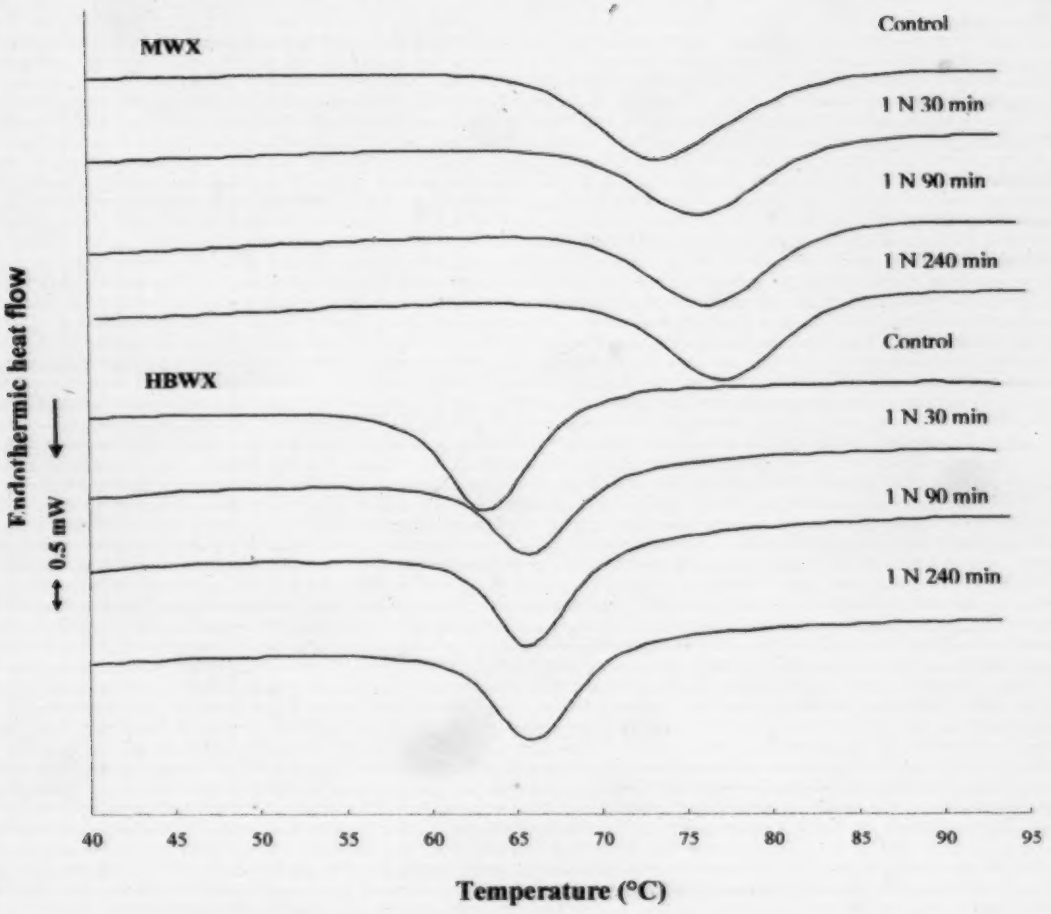

Figure 4.5 DSC thermograms of control and acid treated maize waxy starch (MWX) and hulless barley waxy starch (HBWX).

obtained using a differential scanning calorimeter (DSC), this process of dissociation is associated with a low temperature narrow endotherm usually observed between 50-90°C for most starches, where it represents primarily the melting of amylopectin double helices. Since crystallites within a starch granule vary in their strength, the gelatinization occurs over a wide range of temperature. Jenkins et al. (1994) reported that smaller amylopectin crystallites are less stable and are destroyed first. Cooke and Gidley (1992) reported that gelatinization enthalpy (ΔH) has been shown to reflect loss of molecular (double helical) rather than loss of crystalline order. As discussed earlier, crystallite disorganization during gelatinization of native starches in excess water results in a narrow endotherm, due to swelling of the amorphous regions (bulk and intercrystalline) which exerts a destabilizing effect on the crystallites. This is made possible due to close networking and interaction between the amorphous regions and crystallites. Therefore, the loss of amorphous regions upon acid hydrolysis, as shown above by the data on relative crystallinity (Table 4.1) and swelling factor (Table 4.2), will decrease their destabilizing effect on crystallites. Consequently, in all starches, crystal melting would occur at higher temperatures (T_o and T_p) (Table 4.3) following acid hydrolysis. However, the observed marginal change (Table 4.3) in T_c indicates that during short term acid hydrolysis, only the amorphous regions of native starch granules, which are interconnected with crystallites of low T_o are selectively hydrolyzed.

Moates et al. (1997) demonstrated that amylose crystals from high molecular weight (DP>50) amylose chains are strong in nature and does not melt at low temperatures (usually melt at temperatures >140°C). In this study, the melting

endotherm of amylose crystals formed by partial acid hydrolysis of amylose chains was not observed due to DSC-pan failure at temperatures exceeding 120°C.

In addition, the hydrolysis of inter-crystalline amorphous regions [that are close to amylopectin branch points (as schematically shown in Figure 4.4)] that are connected to amylopectin double helices/crystals would result in double helices/crystals separating from the amorphous network, thereby increasing the melting temperature (possibly > 140°C). Therefore, after acid hydrolysis, these disentangled helices/crystals that were originally part of the amorphous-crystalline network (micelle network), and which may have contributed to the ΔH of gelatinization of native starches would no longer contribute to the gelatinization ΔH of acid treated starches. The observed decrease in enthalpy (Table 4.3) in acid treated HA and WX starches thus stands explained. However, upon acid hydrolysis, the RA starches from both maize and HB starches showed no reduction (Table 4.3) in the enthalpy, indicating RA starches resist acid hydrolysis and undergo minimal loss of their bulk amorphous regions. This is also evident from the low degree of hydrolysis (Figure 4.1 a, b) shown by RA starches.

4.3.6 Chemical reactivity of control and acid treated starches

4.3.6.1 Phosphorylation

The bound phosphorous content (BPC) of control (treated in water without acid) and acid treated (at various time intervals up to 240 min) starches is presented in Table 4.4. Control HA and WX starches from maize and barley had comparable BPC (~2.3%, db), but both had higher BPC than that of RA starches (2.07%, db). Acid pre-treatment of maize and barley starches significantly ($p < 0.05$) influenced the BPC. In general,

treatment up to 90 min enhanced ($p < 0.05$) BPC, but further treatment (up to 240 min) decreased ($p < 0.05$) the BPC. Comparing the highest BPC of maize and barley starches treated with 1 N HCl, the percentage increase (relative to controls) in BPC differed with genotype: MHA and HBHA were 31% at 60 min and 24% at 60 min, respectively, followed by MRA and HBRA at 23% at 60 min and 22% at 90 min, respectively, and MWX and HBWX at 18.4% at 30 min and 18.7% at 90 min, respectively. At higher acid concentration (2.2 N HCl), and with longer treatment times, similar trends in the BPC (i.e. increasing initially and then decreasing) were observed, except for MWX, where only a decrease in BPC was observed. However, the initial increase in BPC after 2.2 N HCl treatment was lower than that seen after 1 N HCl. In addition, after 240 min of acid treatment with 2.2 N HCl, the BPC of HA and WX starches from maize and HB decreased to levels significantly lower than those of control.

The reason for the initial increase in bound phosphorous content is not clear. However, it is postulated that at the initial stages of the treatment, acid molecules come in contact with the starch granule surface, and the resulting hydrolysis would improve surface porosity, thereby facilitating the entry of the phosphorylating reagent into the amorphous domains of the granule. In addition, the hydrolysis of amylose chains and their consequent retrogradation (inter-chain packing) may also clear the path for the phosphorylating reagent to access the potential binding sites (amorphous chains of amylopectin that exist closer to its branch points) in the inter-crystalline amorphous regions. However, the decreased BPC at longer treatment times may be attributed to the loss of amorphous sites due to mechanisms explained earlier in section 4.3.3.

Table 4.4 Bound phosphorous content [1] of control (treated with water) and acid pre-treated maize and hulless barley starches after phosphorylation

	Bound Phosphorous content (%)					
	MHA	**MRA**	**MWX**	**HBHA**	**HBRA**	**HBWX**
Control	2.35 ^{e}	2.06 ^{e}	2.39 ^{b}	2.30 ^{e}	2.08 ^{cd}	2.30 ^{c}
1 N HCl						
30 min	2.67 ^{c}	2.41 ^{b}	2.83 ^{a}	2.52 ^{c}	2.30 ^{b}	2.41 ^{b}
60 min	3.08 ^{a}	2.53 ^{a}	2.76 ^{a}	2.85 ^{a}	2.49 ^{a}	2.69 ^{a}
90 min	3.02 ^{a}	2.51 ^{a}	2.80 ^{a}	2.65 ^{b}	2.55 ^{a}	2.73 ^{a}
120 min	2.81 ^{b}	2.30 ^{c}	2.31 ^{bc}	2.53 ^{c}	2.28 ^{b}	2.27 ^{cd}
240 min	2.45 ^{d}	2.11 ^{de}	2.19 ^{de}	2.39 ^{de}	2.00 ^{d}	2.22 ^{cd}
2.2 N HCl						
30 min	3.10 ^{a}	2.15 ^{de}	2.31 ^{bc}	2.45 ^{cd}	2.29 ^{b}	2.49 ^{b}
60 min	2.91 ^{b}	2.27 ^{cd}	2.35 ^{bc}	2.31 ^{e}	2.11 ^{c}	2.44 ^{b}
90 min	2.82 ^{b}	2.20 ^{cd}	2.26 ^{cd}	2.04 ^{fg}	1.98 ^{d}	2.17 ^{d}
120 min	2.88 ^{b}	2.19 ^{d}	2.15 ^{e}	2.07 ^{f}	2.01 ^{cd}	2.23 ^{cd}
240 min	2.13 ^{f}	2.17 ^{d}	1.75 ^{f}	1.96 ^{g}	2.03 ^{cd}	2.06 ^{e}

[1] All data represent the mean based on two replicate experiments followed by duplicate measurements. Means within a column with different superscripts are significantly different ($p < 0.05$).

Control = starches treated in water, but without acid; MHA = maize high amylose starch; MRA = maize regular starch; MWX = maize waxy starch; HBHA = Hulless barley high amylose starch; HBRA = Hulless barley regular starch and HBWX = Hulless barley waxy starch.

4.3.6.2 Cationization

The bound nitrogen content (BNC) of control (treated with water) and acid treated starches is presented in Table 4.5. The BNC of control starches did not differ significantly among the genotypes of maize or barley. Acid pre-treatments both at 1N and 2.2 N had no significant influence on BNC, for an obvious reason. Cationization is done under highly basic conditions (> pH 12), a chemical environment that promotes a high degree of starch swelling due to extreme disruption of intramolecular hydrogen bonding by the hydroxyl ion. Therefore, any impact of acid pre-treatment to predispose starch granules to penetration for cationization is insignificant compared to starch swelling during the alkaline conditions of the cationization reaction.

4.4 Conclusions

The study has shown that the partial acid hydrolysis of native starches influences their physicochemical properties such as RC, SF, gelatinization parameters and chemical reactivity. The data in general indicated that the changes in the aforementioned physicochemical properties are primarily due to the loss of amorphous regions caused by acid hydrolysis, which in turn is primarily responsible for the observed changes in chemical reactivity of acid treated maize and HB starches. The observed enhancement in the reactivity of acid treated starches towards phosphorylation is beneficial to starch industry by decreasing the amount of phosphorylating reagent needed for starch phosphorylation.

Table 4.5 Bound nitrogen content [1] of control (treated with water) and acid pre-treated maize and hulless barley starches after cationization

	Bound Nitrogen content (%)					
	MHA	**MRA**	**MWX**	**HBHA**	**HBRA**	**HBWX**
Control	0.37 [a]	0.32 [a]	0.33 [a]	0.29 [a]	0.25 [a]	0.32 [a]
1 N HCl						
30 min	0.38 [a]	0.31 [a]	0.32 [a]	0.28 [a]	0.23 [a]	0.29 [a]
60 min	0.37 [a]	0.35 [a]	0.31 [a]	0.28 [a]	0.25 [a]	0.30 [a]
90 min	0.38 [a]	0.30 [a]	0.35 [a]	0.29 [a]	0.26 [a]	0.31 [a]
120 min	0.39 [a]	0.30 [a]	0.33 [a]	0.26 [a]	0.22 [a]	0.27 [a]
240 min	0.38 [a]	0.33 [a]	0.30 [a]	0.27 [a]	0.28 [a]	0.35 [a]
2.2 N HCl						
30 min	0.36 [a]	0.30 [a]	0.30 [a]	0.26 [a]	0.25 [a]	0.30 [a]
60 min	0.35 [a]	0.33 [a]	0.31 [a]	0.27 [a]	0.27 [a]	0.30 [a]
90 min	0.34 [a]	0.35 [a]	0.31 [a]	0.27 [a]	0.24 [a]	0.34 [a]
120 min	0.37 [a]	0.35 [a]	0.33 [a]	0.27 [a]	0.27 [a]	0.30 [a]
240 min	0.35 [a]	0.32 [a]	0.32 [a]	0.27 [a]	0.26 [a]	0.29 [a]

[1] All data represent the mean based on two replicate experiments followed by duplicate measurements. Means within a column with different superscripts are significantly different ($p < 0.05$).

Control = starches treated in water, but without acid; MHA = maize high amylose starch; MRA = maize regular starch; MWX = maize waxy starch; HBHA = Hulless barley high amylose starch; HBRA = Hulless barley regular starch and HBWX = Hulless barley waxy starch.

4.5 References

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Chapter 5

Summary and conclusions

The objectives of this thesis research were as follows: 1) to obtain highly purified starch isolates from cultivars of regular (~24% amylose), waxy (~5% amylose) and high amylose (~35% amylose) hulless barley (HB) grains by combining a aqueous-alcoholic and aqueous fractionation procedures; 2) to compare the morphology and physicochemical properties of native and partially acid (1 N and 2.2 N HCl) hydrolyzed (up to 4 h) HB and maize starches and 3) to determine the reactivity of native and partially hydrolyzed HB and maize starches towards phosphorylation and cationization.

The starch isolates, which were mainly composed of large granules, showed high purity (> 98%, w/w) with trace amounts of protein (< 0.3%, w/w) and beta-glucan (< 0.1%, w/w). The yield (%, w/w) of starch isolates, fiber concentrates and protein concentrates obtained during fractionation ranged between 22-39% (w/w), 17-32% (w/w) and 32-46 % (w/w), respectively. The fiber concentrate contained beta-glucan (17-23%, w/w), starch (25-39%, w/w), and protein (11-14%, w/w). The starch content in the protein concentrate ranged between 41-55% (w/w) and was composed mainly of small starch granules. The recovery rates for starch, protein and beta-glucan were over 91% (flour dry weight basis) for the total of three fractions collected. The starch extraction efficiency was in the range 38-59%, w/w. The physicochemical properties (viscosity and gelatinization characteristics), X-ray patterns and crystallinity of the isolated starches

were comparable to those of other barley starches that have been extracted by traditional aqueous techniques.

The degree of acid hydrolysis in the hulless barley starches after a 4 h of treatment with 1 N and 2.2 N HCl was in the range of 1.4-2% and 2.1-3%, respectively. The surface characteristics of all hulless barley starches remained unchanged upon acid hydrolysis. At both acid concentrations, the relative crystallinity of regular barley starches remained unchanged on hydrolysis. However, those of waxy and high amylose starches increased. This was attributed to: 1) additional interactions between double helices resulting from the hydrolysis of the amorphous regions near the branch points of amylopectin; 2) the loss of amylose chains in the bulk amorphous regions; and 3) retrogradation of the hydrolyzed amylose chains.

The swelling factor (SF) of all native hulless barley starches followed the order WX > RA > HA. Acid hydrolysis decreased SF in all starches. The extent of this decrease (highest in the waxy starches) was inversely proportional to acid strength and hydrolysis time. The reduction in SF was attributed to hydrolysis of the amorphous regions and creation of new crystalline regions.

The gelatinization temperature range and the enthalpy (ΔH) of the native starches followed the order: HA > WX > RA and WX > RA ~ HA, respectively. Acid hydrolysis significantly increased T_o and T_p, but increased T_c only marginally. The ΔH decreased significantly in WX and HA starches, but remained unchanged in RA starch. The above changes were explained as being due to hydrolysis of the inter-crystalline amorphous regions near the amylopectin branch points.

Acid hydrolysis first increased the extent of phosphorylation in all starches. The percentage increase varied among the genotypes (HA > RA > WX). Hydrolysis beyond the certain extent decreased phosphorylation in all starches. The increase in phosphorylation was attributed to hydrolysis of starch chains that may have been present at the granular surface. Whereas, the decrease in phosphorylation at over-extended hydrolysis may have been due to extensive hydrolysis of starch chains in the bulk and inter crystalline amorphous regions.

The acid hydrolysis had no influence on the extent of cationization. This was due to the alkaline conditions used in cationization increasing granular swelling to such an extent that it negates any changes within the amorphous regions due to acid hydrolysis.

The present study has demonstrated an improved protocol for HB starch purification that solves a number of technical challenges commonly encountered during traditional aqueous processing. These challenges are mainly due to the increase in slurry viscosity because of beta-glucan hydration. Since beta-glucan is removed by an aqueous-alcoholic (50% alcohol) step at the initial stages of the protocol (beta-glucan does not hydrate in aqueous-alcoholic media), the second step involving the aqueous processing becomes easier. Therefore, the suggested new protocol not only generates a high value beta-glucan fiber concentrate (a nutraceutical component for food and supplement industry), but also provides an efficient process for producing high purity HB starches. Furthermore, the partial acid hydrolysis would provide a means of reducing the cost of phosphorylation by increasing the reactivity of the starch granule towards the phophorylating reagent. As a future direction for this research, it will be interesting to determine how partially acid hydrolyzed starches would behave towards other chemical

modification techniques such as acetylation, propylation, etc and physical modification techniques such as annealing and heat-moisture treatment.